THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

NOVEMBER, 1957

ORIGINAL ARTICLES

METABOLIC STUDIES IN PATIENTS RECEIVING LIPID MOBILIZER HORMONE*

By Chris J. D. Zarafonetis, M.D.
CLINICAL PROFESSOR OF MEDICINE

Gladys M. Miller, M.D.
INSTRUCTOR IN MEDICINE

Joseph Seifter, M.D.
DIRECTOR, WYETH INSTITUTE FOR MEDICAL RESEARCH

David Baeder, Ph.D.
PHARMACOLOGIST, WYETH INSTITUTE FOR MEDICAL RESEARCH

Ralph M. Myerson, M.D.
ASSISTANT CHIEF, MEDICAL SERVICE, VETERANS ADMINISTRATION HOSPITAL

AND

William A. Steiger, M.D.
ASSISTANT PROFESSOR OF MEDICINE

(From the Department of Medicine, Temple University School of Medicine, Philadelphia, Pennsylvania; the Wyeth Institute for Medical Research, Radnor, Pennsylvania; and the Veterans Administration Hospital, Philadelphia, Pennsylvania)

Seifter and Baeder[8,9] reported the occurrence of an extractable lipid mobilizing hormone (LM) in the plasma of several animal species, including man. They demonstrated that dialyzates of plasma from animals administered cortisone, subjected to stress or to the nephrotic syndrome, inhibited the delactescing action of the heparin clearing factor. It was further shown that the injection of such dialyzates into sensitized animals produced hyperlipemia. The inhibition of heparin clearing factor by cortisone administration has been confirmed by Meng, Hollet and Cole[4] and for stress by Robinson and Harris[5]. The presence of such an inhibitor of the delactescing action of heparin clearing factor in certain clinical hyperlipemias has been reported by Klein and Lever[3]. More recently, Seifter and Baeder[11] have recovered LM from the posterior pituitary of hogs indicating that this hor-

*Presented, in part, at the meeting of the Central Society for Clinical Research, Chicago, Illinois, November 9, 1956.

mone is either produced or stored at that site. These workers had previously reported that the pituitary was essential for the release of LM in rats subjected to a variety of stresses[10], including the administration of protamine, as has been confirmed by Schotz and Page[7]. In this connection, it should be noted that Bally and Numa[1] found that the pituitary was essential for the production of hyperlipemia in rats subjected to the nephrotic syndrome.

The first studies on the effects of this lipid mobilizing hormone in man were performed by Steiger *et al.*[13] and Zarafonetis *et al.*[15]. Preliminary observations showed a marked elevation of the plasma cholesterol, fatty acids, and lipid phosphorus resulted from a single intravenous injection of LM into each of 25 fasting patients. In view of these striking responses, it became of interest to extend the observations to subjects receiving LM over prolonged periods, and to determine the fate of the mobilized lipids if possible. Accordingly, metabolic studies were undertaken in patients who were given daily injections of LM for 2 weeks. The findings in these studies form the basis of the present report.

Material and Methods. Eight adult male patients who required prolonged hospitalization for chronic diseases were selected from the Medical Service for study on the Metabolic Unit of the Veterans Administration Hospital. Their respective ages, weights, and diagnoses are listed in Table 1. The subjects were studied in pairs, each pair remaining on the experimental program for 4 weeks but with subsequent tests in some instances as indicated below.

DIET. A low fat diet was fed to 6 of the subjects for 4 weeks. It consisted of a liquid formula diet which provided a daily average of 2230 calories derived from 381 gm. carbohydrate, 166 gm. protein, and 4.8 gm. fat. The first and fourth weeks were control periods, while daily intravenous injections of LM were given during the second and third weeks to 4 of the subjects (Table 1). In order to assess the effect of the experimental diet itself, the other 2 individuals (J.J. and C.L.) who were on this regimen received only placebo injections of physiological salt solution during the second and third weeks. However, at the conclusion of the experimental period, the response of both subjects to an intravenous injection of LM (5 mg. per kg.) was determined.

TABLE 1.—PATIENTS STUDIED

Patient	*Age*	*Sex*	*Weight*	*Diagnosis*	*Avg. Daily Fat Intake*	*Injected with*
1. P. G.	29	M	50 kg.	Epilepsy	4.8 gm.	LM*
2. R. H.	62	M	62 kg.	Multiple sclerosis	4.8 gm.	LM
3. B. P.	42	M	48 kg.	Multiple sclerosis	4.8 gm.	LM
4. G. S.	45	M	50 kg.	Rheum. arthritis	4.8 gm.	LM
5. J. J.	37	M	58 kg.	Sickle cell anemia	4.8 gm.	Physiol. NaCl
6. C. L.	48	M	107 kg.	Early cirrhosis	4.8 gm.	Physiol. NaCl
7. J. T.	34	M	75 kg.	Multiple sclerosis	153.0 gm.	LM
8. J. S.	27	M	60 kg.	Rheum. arthritis	99.0 gm.	LM

* LM = lipid mobilizer obtained in dialyzate of plasma from cortisone treated horses.

Two other patients (J.T. and J.S.) were placed on another dietary regimen intended to provide a high fat intake. Subject J.T. was able to ingest a daily average of 153 gm. fat, 126 gm. protein, and 188 gm. carbohydrate for a total of 2630 calories. J.S. consumed, on average, 2061 calories a day, obtained from 99 gm. fat, 90 gm. protein, and 202 gm. carbohydrate. Both of these patients received daily intravenous injections of LM during the second and third weeks of the experimental period. In addition, after these individuals had resumed the regular hospital diet for a few days, they were fasted over-

night and re-tested with an intravenous injection of LM (5 mg. per kg.).

DOSAGE OF LM EMPLOYED. The lipid mobilizer used in these studies was prepared by dialysis of citrated plasma from cortisone treated horses as previously noted (Steiger *et al.*[13] and Zarafonetis *et al.*[15]). Therefore, in addition to the active principle, certain dialyzable salts and other substances were present in the experimental material, and account for the relatively large weight (milligrams) of the doses used.* Thus, 1 mg. LM per kg. was administered, intravenously, on the first day of the 2-week injection period to each of 6 subjects in this study. On the second day, the dose of LM was increased to 3 mg. per kg., and then to 5 mg. per kg. on the third day. The dosage was continued at this level for patients, R.H., G.S., and J.T. throughout the remaining 11 days. In the case of P.G., B.P., and J.S., however, the dose was increased and maintained at 10 mg. LM per kg. daily during the second week.

TESTS PERFORMED. Twenty-four-hour urine and feces collections were made throughout the test period. The urines were analyzed for cholesterol (digitonide-Liebermann-Burchard material) and nitrogen content (Kjeldahl). Cholesterol (digitonide-Liebermann-Burchard material), fatty acids (Stoddard and Drury[14]), lipid phosphorus (Fiske and SubbaRow[2]), and nitrogen determinations were made on dried aliquots of the 24-hour fecal excretions. Fasting blood specimens were analyzed for cholesterol (Schoenheimer and Sperry[6]), fatty acids (Stoddard and Drury[14]), and lipid phosphorus (Fiske and SubbaRow[2]), on the first third, and fifth day of each week. The above determinations were all made at the laboratories of the Wyeth Institute for Medical Research, Radnor, Pa.

The following clinical and laboratory tests were performed at the V.A. Hospital: Serum electrolytes (Na, K, Cl), carbon dioxide blood glucose, and serum amylase were checked on the first day of each week, as were the erythrocyte and leukocyte counts, differential, hemoglobin, and hematocrit. Serum proteins and the albumin-globulin ratio were determined on the second day of each week. Prothrombin time, bilirubin, alkaline phosphatase, cephalin flocculation, thymol turbidity, and lipase determinations were made weekly (on the fourth day). Electrocardiograms were taken on the third day of each week, while the basal metabolic rate and respiratory quotient were measured on the third and fifth days of every week of the study. Body weight, temperature, and blood pressure were recorded daily.

Results. 1. EFFECTS OF LOW FAT DIET ALONE. a. *Plasma lipids.* The effect of the formula diet itself was studied in patients, J.J. and C.L., who were given placebo injections of physiological salt solution instead of LM during the experimental period. Figure 1 depicts the total cholesterol, total fatty acids, and lipid phosphorus values obtained in subject C.L. during the test period. As can be seen, these plasma lipid values declined slightly and insignificantly. Similar changes were observed in the plasma lipids of J.J. At the conclusion of the study period, the response of both subjects to an intravenous injection of LM (5 mg. per kg.) was determined. Baseline values for C.L. were as follows: total cholesterol, 172 mg. per 100 ml.; fatty acids, 180 mg. per 100 ml.; and lipid phosphorus, 6.0 mg. per 100 ml. Four hours after the injection of LM, the plasma cholesterol rose to 396 mg. per 100 ml., the fatty acids to 412 mg. per 100 ml., and lipid phosphorus to 11.2 mg. per 100 ml. Comparable changes were noted after injection of LM into J.J.

b. *Fecal lipids.* The low fat diet resulted in a marked decrease from baseline values of the fecal fatty acid and fecal lipid phosphorus. Fecal cholesterol excretion, however, remained relatively constant (Fig. 2).

c. *Urinary cholesterol.* The 24-hour urine output of cholesterol was minimal, averaging 2 to 3 mg. daily (Fig. 2).

d. *Nitrogen excretion.* A fall in fecal and urinary nitrogen excretion was noted in all 6 subjects during the early period of low fat intake, and both stabilized at the lower levels for the

*Recent evidence indicates that the pure LM hormone is effective in man in microgram doses (Seifter and Baeder[12] and Zarafonetis *et al.*[16]).

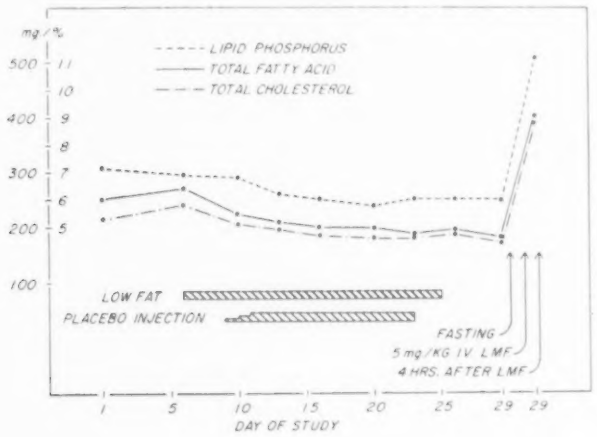

Fig. 1

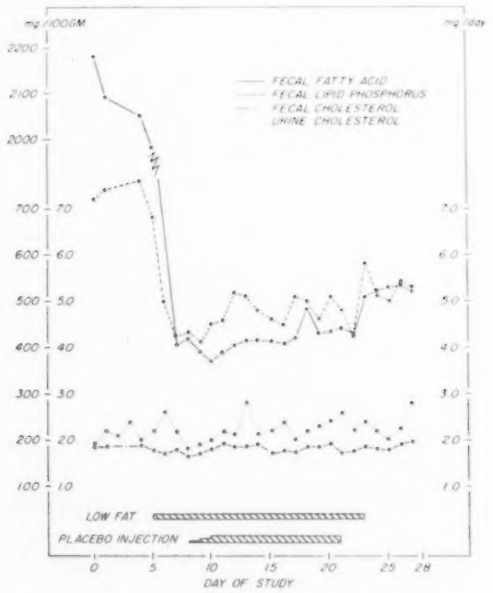

Fig. 2

Fig. 1.—Plasma lipid levels in subject C.L., a white male with early cirrhosis of liver; low fat diet and placebo intravenous injections of physiological saline solution. Note acute response to injection of LM at end of study period.

Fig. 2.—Fecal and urinary lipid excretions of subject, C.L. Note decrease in fecal fatty acids and lipid phosphorus coincident with low fat diet.

remainder of the dietary period. The findings in C.L. and J.J. were similar to those obtained in the LM treated patients (Figs. 9 and 10) and are, therefore, not separately illustrated.

2. EFFECTS OF LM ADMINISTRATION TO SUBJECTS ON LOW FAT DIET. a. *Plasma lipids.* A striking and sustained increase in plasma cholesterol, fatty acids, and lipid phosphorus resulted in all 4 subjects receiving intravenous injections of LM. These responses are graphically shown in Figs. 3, 4, 5, and 6. It can be seen that the lipid values remained

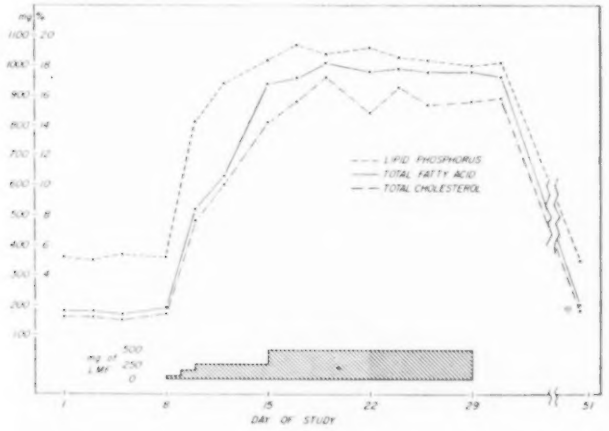

Fig. 3

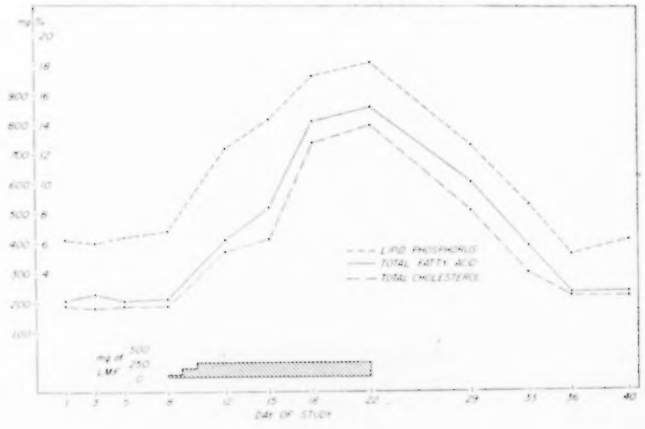

Fig. 4

Fig. 3.—Fasting plasma lipid levels of P.G., before, during and after injection of lipid mobilizing hormone.

Fig. 4.—Fasting plasma lipid values of G.S., low fat diet and indicated LM administration.

elevated for a week or more after the administration of LM had been discontinued.

b. *Fecal lipids.* Fecal fatty acids and lipid phosphorus excretion decreased in a manner similar to that observed in the dietary control cases. However, fecal cholesterol (digitonide-precipitated Liebermann-Burchard reacting material) increased from approximately 200 mg. per 100 gm. of dried feces to as much as 600 mg. per

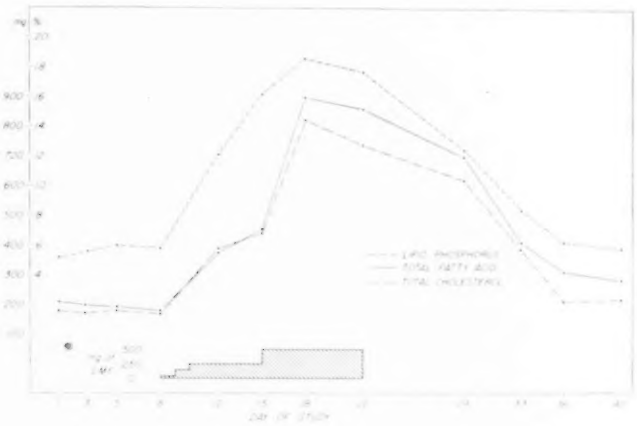

Fig. 5

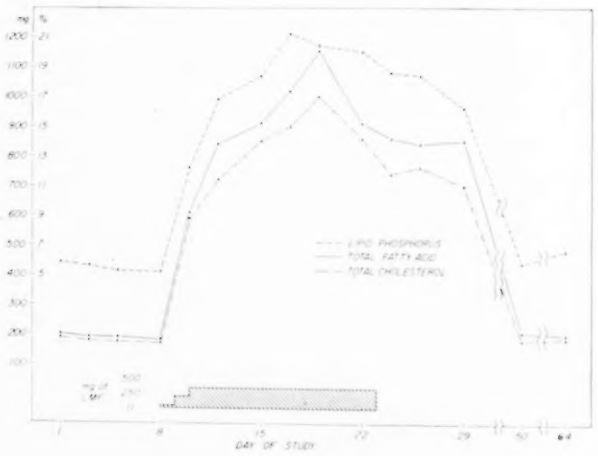

Fig. 6

Fig. 5.—Fasting plasma lipids of B.P., subject on low fat diet. Note apparent stepwise elevation of values with increased increment of LM.

Fig. 6.—Fasting plasma lipid levels of R.H., patient with multiple sclerosis, on low fat diet. This subject was on isoniazid at time of LM administration.

100 gm. of dried feces (Figs. 7 and 8).

c. *Urinary cholesterol.* A definite increase in urinary cholesterol excretion was observed following administration of LM. However, the cholesterol loss in the urine was raised only from 1 to 2 mg. per day to 4 to 6 mg. daily (Figs. 7 and 8).

d. *Nitrogen excretion.* The excretion of urinary and fecal nitrogen was decreased as a result of the low fat diet and showed no further change

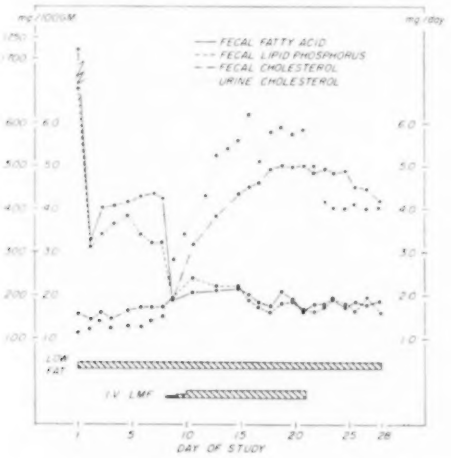

Fig. 7

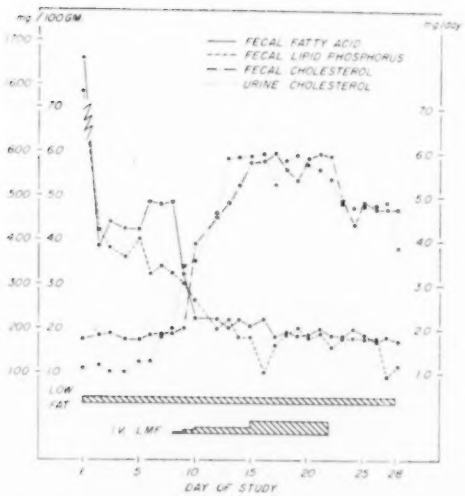

Fig. 8

Fig. 7.—Increased urinary and fecal cholesterol excretion of subject, G.S. following LM administration. Contrast with control subject, C.L., in Fig. 2.

Fig. 8.—Increased urinary and fecal cholesterol excretion of subject, B.P., following LM administration. Contrast with control subject, C.L., in Fig. 2.

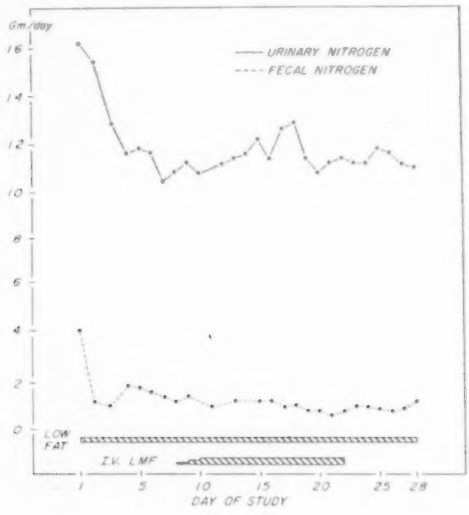

Fig. 9

Fig. 10

Fig. 9.—Initial decrease in urinary and fecal nitrogen excretion of subject, G.S., coincident with start of low fat diet.

Fig. 10.—Initial decrease in urinary and fecal nitrogen excretion of subject, B.P., coincident with start of low fat diet.

attributable to the administration of LM (Figs. 9 and 10).

3. EFFECT OF LM ADMINISTRATION TO SUBJECTS ON HIGH FAT DIET. a. *Plasma lipids.* Both subjects had some pre-LM rise in plasma lipid values presumably due to dietary factors. The administration of LM failed to elicit any further increase in these plasma components. Of interest in both subjects is the definite decline of plasma lipid concentration during the period immediately

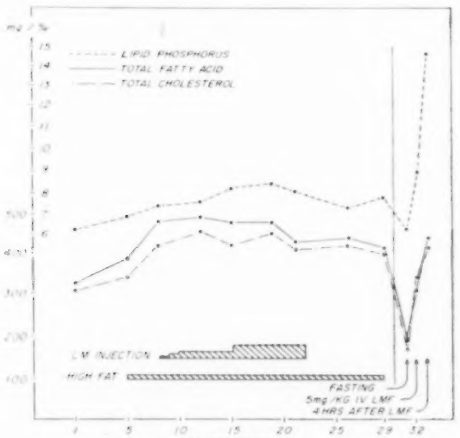

Fig. 11

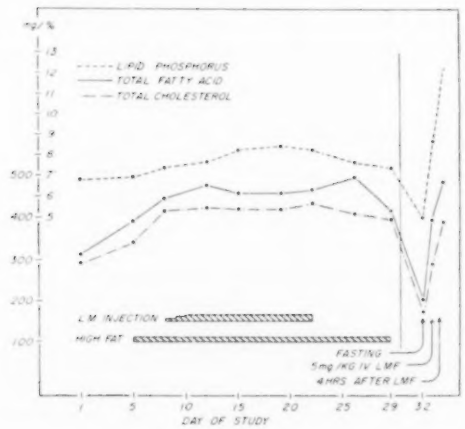

Fig. 12

Fig. 11.—Fasting plasma lipid values of J.S., patient with rheumatoid arthritis, whose diet contained daily average of 99 gm. fat. Note lack of response to LM administration, the sharp decline in plasma lipids upon cessation of special diet, and the acute response to LM when fasting.

Fig. 12.—Fasting plasma lipid values of J.T., patient with multiple sclerosis, whose diet provided an average of 153 gm. fat daily. Note similarity of findings to those of J.S. (Fig. 11).

following cessation of the experimental diet. As can be seen in Figs. 11 and 12, the lipids decreased to levels even lower than the base-line values. At this point, both subjects were tested after an overnight fast and found to respond promptly to an injection of LM.

b. *Fecal lipids.* The fecal extraction of fatty acids was definitely increased, while minor increases occurred in lipid phosphorus and cholesterol excretion (Fig. 13).

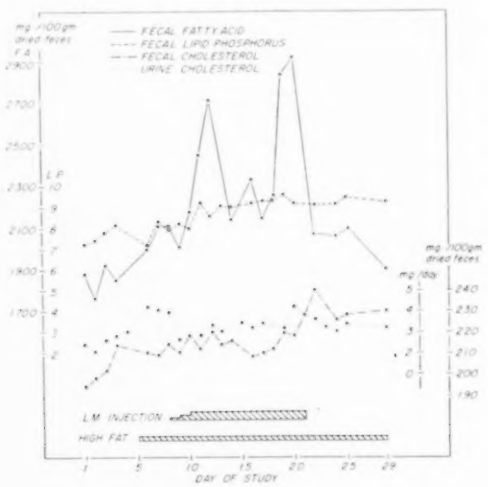

Fig. 13

Fig. 13.—Fecal and urinary excretion of lipids by J.T., patient on high fat diet. Note increased fecal fatty acid output in contrast to subjects on low fat diet. The fecal lipid phosphorus is fairly constant. There is no sharp rise in fecal and urinary cholesterol excretion as was observed following LM administration to subjects on a low fat diet.

c. *Urinary cholesterol.* Excretion of urinary cholesterol remained in a range of 2 to 4 mg. daily (Fig. 13).

d. *Nitrogen excretion.* In contrast to the subjects on the low fat diet, these patients showed no significant decline in fecal nitrogen excretion, and only a small initial fall in urinary nitrogen excretion.

4. CLINICAL AND LABORATORY TESTS. With one exception, to be noted below, there were no significant changes in urinalyses, serum electrolytes, blood glucose, serum proteins, hemograms, liver function tests, electrocardiograms, and blood pressure. The exception was R.H., a 62-year-old man with multiple sclerosis, who was also receiving isoniazid, 100 mg., t.i.d. This subject developed periods of confusion during the second week of LM administration, and signs of a mild hepatitis. At this time, the bromsulfalein test revealed 12.5% dye retention after 45 minutes. The alkaline phosphatase rose to 17.3 units from a base-line of 8.0 units, and the cephalin flocculation test became 3 plus (base-line 1 plus). The serum glutamic-oxalacetic transaminase, which was 64 units at the beginning of the study period, reached a high of 192 units at this time. LM and isoniazid were discontinued and the values soon returned to base-line levels. One pa-

tient, B.P., gained 13 lbs. while on the metabolic unit, but none of the other subjects showed any significant weight change. There was some variation in the respiratory quotient results, but these were inconsistent and did not appear to be influenced by the administration of LM.

Discussion. The intravenous administration of lipid mobilizer to subjects on a low fat diet resulted in a remarkable increase in plasma cholesterol, fatty acids, and lipid phosphorus. This effect was sustained as long as the injections were continued. After LM administration was stopped, however, the lipid values gradually declined to pre-injection levels in a week or two.

During the period of LM induced hyperlipemia, there was a definite increase in urinary and fecal cholesterol excretion. However, the cholesterol loss in the urine was raised only from 1 to 2 mg. per day to 4 to 6 mg. daily. At the same time, fecal cholesterol, that is, digitonide-precipitated Liebermann-Burchard reacting material, increased from approximately 200 mg. per 100 gm. of dried feces to as much as 600 mg. per 100 gm. of dried feces.

The subjects on the low-fat diet regimen (4.8 gm. per day), also exhibited decreased excretion of fecal fatty acids and lipid phosphorus, and of fecal and urinary nitrogen. These findings, however, were attributable to the diet itself since the same changes were noted in the patients who received placebo injections as well as in those who were given LM. Thus, of the observed effects, the sustained hyperlipemia and increased urinary and fecal cholesterol excretion resulted from the injection of LM. While the increase in fecal and urinary cholesterol excretion is definite in these subjects, it should be evident that it accounts only for a fraction of the total amount mobilized. The fate of the remainder, as well as of the other plasma lipid components, remains to be explained. In any event, there is nothing in these observations to suggest that LM enhances the metabolism of lipids in addition to its mobilization of them.

It should be noted further that the above indicated effects were the only changes detected in these subjects. Serum electrolytes, glucose, proteins, hemograms, and respiratory quotients showed no significant change. One subject gained 13 lbs. but he had had an acute weight loss prior to admission to the metabolic study. Another, who was also receiving isoniazid for multiple sclerosis, did manifest clinical and laboratory evidence of impairment of liver function. This subsided shortly after isoniazid and LM administration were discontinued.

In contrast to the findings in the low-fat diet subjects are the observations in the 2 patients who were fed a daily average of 99 gm. and 153 gm. of fat, respectively. Both of these individuals had some pre-LM rise in plasma lipid values, apparently due to the diet. The daily intravenous injection of LM into these subjects, however, did not elicit any further increase in the plasma lipid components. Of interest in both cases was the observation of a sharp decline in the concentration of plasma lipids during the period immediately succeeding the experimental diet. It seems reasonable to assume that a lipid removal or clearing mechanism had been activated, presumably by the relatively high fat intake, and that this mechanism continued to operate, thereby reducing the plasma lipids to values even lower than had been found at the beginning of the experimental period.

It is also of interest that neither of these subjects exhibited the increase in fecal and urinary cholesterol excretion which was induced by LM in the low-

fat diet patients. That these subjects were not otherwise refractory to LM is evident from the plasma lipid response observed when they were tested a few days after conclusion of the special feeding program.

These observations have extended and added information concerning the effects of lipid mobilizer in humans. On the basis of these and other studies, it would appear that certain metabolic posture or postures must be present for the lipid mobilizing effect either to be elicited or manifest, or both. A diet providing a high fat intake, for example, blocks the response to LM, while a greatly restricted fat intake does not. Studies designed further to define these conditions and to determine clinical relationships of possible LM derangements are underway in these clinical and experimental laboratories.

Summary and Conclusions. 1. Lipid mobilizer was administered daily for 2 weeks to 4 patients maintained on a diet which provided only 4.8 gm. fat daily. In these individuals, there resulted a striking elevation of plasma cholesterol, fatty acids, and lipid phosphorus, and a definite increase in fecal and urinary cholesterol excretion.

2. These effects were not observed when LM was administered to 2 other subjects who were maintained on diets providing 99 gm. and 153 gm. of fat daily, respectively.

3. Response to LM appears to depend on appropriate metabolic conditions at the time of injection. A diet very low in fat content permits a marked hyperlipemia following LM administration, while a high fat diet prevents such a response to similar amounts of LM.

ACKNOWLEDGMENTS: The authors wish to thank Dr. Doris F. Campbell and Miss Mae Purcell, of the Veterans Administration Hospital, Philadelphia, and W. H. Beckman, J. Dachiw, Joan Howitz, Marie Klenk, and E. E. Livingston, Jr., of the Wyeth Research Institute, Radnor, Pa., for technical assistance during the course of this study.

REFERENCES

1. **Bally, P.**, and **Numa, S.**: Abstr., Program, 49th Annual Meeting, Am. Soc. Clinical Investig., p. 8, May 6, 1957.
2. **Fiske, C. H.**, and **SubbaRow, Y.**: J. Biol. Chem., **66**, 375, 1925.
3. **Klein, E.**, and **Lever, W. F.**: Proc. Soc. Exper. Biol. & Med., **95**, 565, 1957.
4. **Meng, H. C.**, **Hollet, C.**, and **Cole, W. E.**: Am. J. Physiol., **179**, 314, 1954.
5. **Robinson, D. S.**, and **Harris, C. M.**: Biochem. J., **66**, 18, 1957.
6. **Schoenheimer, R.**, and **Sperry, W. M.**: J. Biol. Chem., **106**, 745, 1934.
7. **Schotz, M. C.**, and **Page, I. H.**: Abstr., Circulation, **16**. 515, 1957.
8. **Seifter, J.**, and **Baeder, D. H.**: Proc. Soc. Exper. Biol. & Med., **86**, 709, 1954.
9. Iidem! Ibid., **91**, 42, 1956.
10. Iidem: Ibid., **93**, 63, 1956.
11. Iidem: Ibid., **95**, 318, 1957.
12. Iidem: Ibid., **95**, 469, 1957.
13. **Steiger, W. A.**, **Zarafonetis, C. J. D.**, **Miller, G. M.**, **Seifter, J.**, and **Baeder, D. H.**: Am. J. Med. Sci., **232**, 605, 1956.
14. **Stoddard, J. L.**, and **Drury, P. E.**: J. Biol. Chem., **84**, 741, 1929.
15. **Zarafonetis, C. J. D.**, **Miller, G. M.**, **Steiger, W. A.**, **Seifter, J.**, **Baeder, D. H.**, and **Myerson, R. M.**: Abstr. J. Lab. & Clin. Med., **48**, 961, 1956.
16. **Zarafonetis, C. J. D.**, **Miller, G. M.**, **Seifter, J.**, and **Baeder, D. H.**: To be published.

(Interlingua on page 560)

SULFAMETHOXYPYRIDAZINE: CONCENTRATIONS IN BLOOD AND URINE DURING ORAL ADMINISTRATION OF VARIOUS DOSAGES*

By Maxwell Finland, M.D.
ASSOCIATE PROFESSOR OF MEDICINE, HARVARD MEDICAL SCHOOL; ASSOCIATE DIRECTOR, THORNDIKE MEMORIAL LABORATORY, AND PHYSICIAN IN CHIEF, FOURTH MEDICAL SERVICE, BOSTON CITY HOSPITAL

Wilfred F. Jones, Jr., M.D.
ASSISTANT IN MEDICINE, HARVARD MEDICAL SCHOOL; RESEARCH FELLOW, THORNDIKE MEMORIAL LABORATORY

Mohsen Ziai, M.D.
RESEARCH FELLOW, HARVARD MEDICAL SCHOOL AND THORNDIKE MEMORIAL LABORATORY

AND

Gilbert R. Cherrick, M.D.
ASSISTANT IN MEDICINE, HARVARD MEDICAL SCHOOL; RESIDENT PHYSICIAN, SECOND MEDICAL SERVICE, BOSTON CITY HOSPITAL

(From the Thorndike Memorial Laboratory, Second and Fourth (Harvard) Medical Services, Boston City Hospital and the Department of Medicine, Harvard Medical School, Boston, Massachusetts)

Sulfamethoxypyridazine† is a new sulfonamide with unusual properties. On a dosage basis it was found to be several times as potent as sulfisoxazole (Gantrisin) and sulfadimidine (Elkosin) against experimental infections of mice with several susceptible organisms. On a basis of blood levels it was also shown to be more effective than these agents, its antibacterial activity being equivalent to that of sulfadiazine or the triple sulfonamide mixture containing equal amounts of sulfadiazine, sulfamerazine and sulfamethazine (Litchfield[10]). It has low toxicity in animals (Litchfield[10], Roepke, Maren and Mayer[13]). It is well absorbed after oral administration and diffuses well into body fluids, at least as evidenced by its good diffusion into the cerebrospinal fluid (Boger, Strickland and Gyle[1]).

Sulfamethoxypyridazine is of particular interest because its free form is very slowly excreted in the urine, yielding higher and better sustained levels in the plasma than any of the other known and useful antibacterial sulfonamides (Boger, Strickland and Gyle[1], Foerster *et al.*[3], Frisk and Wassen[4], Grieble and Jackson[5], Jones and Finland[6], Jones *et al.*[7], Litchfield[10], Nichols and Finland[11], Nichols, Jones and Finland[12], and Roepke, Maren and Mayer[13]). The conjugated form is excreted into the urine much more rapidly than the free form so that only a small proportion of the drug found in the plasma is in conjugated form (Boger, Strickland and Gyle[1], Foerster *et al.*[3], Frisk and Wassen[4], Litchfield[10], Nichols and Finland[11], Nichols, Jones and Finland[12], and Roepke, Maren and Mayer[13]). Both the free and conjugated

*Aided by a grant (E–23) from the National Institutes of Health.

†The sulfamethoxypyridazine was generously supplied under the trade-mark Kynex by Lederle Laboratories Division, American Cyanamid Co., Pearl River, N.Y.

forms are highly soluble in urine (Litchfield[10] and Roepke, Maren and Mayer[13]). This agent has been used successfully and was well tolerated in early trials for the prevention of streptococcal infections and rheumatic fever (Lepper, Simon and Marienfeld[9]) and in the prevention and treatment of some urinary tract and other infections (Frisk and Wassen[4], Grieble and Jackson[5], Jones and Finland[6], Nichols and Finland[11], Ross, Ahrens and Zaremba[14], and Walker and Hamburger[15]). The present paper deals with observations on levels of sulfamethoxypyridazine in blood and urine during oral administration of various dosages and provides data which may aid in the choice of a proper dosage regimen for optimum clinical application of this potentially useful agent.

Materials and Methods. Single doses of 4.0 and 3.0 gm. were given to normal adult males; other dosage regimens were employed in hospitalized adult patients, many of whom were elderly and had infections of the urinary tract for which they had not recently received any sulfonamide drugs. None of these patients had gross impairment of renal function and the concentrations of nonprotein nitrogen in their blood was within normal limits for such patients. The sex, ages and weights of the patients included in the study of the various dosage regimens are listed in Table 1. The doses were given at stated times each day, and oxalated venous blood was obtained just before and at appropriate intervals after each dose. Complete collections of urine were made for detailed studies of absorption and excretion of the single 4.0 and 3.0 gm. doses (Nichols and Finland[11] and Nichols, Jones and Finland[12]), during the other dosage regimens, specimens of urine voided at the time when the blood was drawn were collected from most of the patients. Concentration of free and total sulfamethoxypyridazine in the blood and urine were assayed by the method of Bratton and Marshall[2]. The chemical determinations were carried out by Miss Ellen J. Doyle and Miss Mary I. Kendrick.

Results. Some of the data obtained in the subjects who were given single doses of 4.0 to 3.0 gm. of sulfamethoxypyridazine have been reported in detail elsewhere (Nichols and Finland[11], Nichols, Jones and Finland[12]); these included concentrations of the drug in plasma and urine, distribution between plasma and blood cells, renal clearances and total urinary recoveries. The levels in blood and urine during repeated doses of 0.5 gm. every 12 hours have also been presented, and the blood levels from this dosage were found to be comparable with those obtained when the chemically related sulfachloropyridazine was given to similar patients in doses of 1 gm. every 6 hours (Jones and Finland[6], and Jones *et al.*[7]). Levels in plasma and urine during administration of 1.0 gm. every 48 hours were also included with an earlier report (Nichols and Finland[11]).

TABLE 1.—SEX, AGE AND WEIGHT OF RECIPIENTS OF VARIOUS DOSAGES

Dosage Regimen	*Sex*	*Ages (yrs.)*	*Weights (kg.)*
4.0 gm., single dose	6M	24, 24, 25, 26, 34, 36	65, 75, 75, 80, 85, 85 (Av. 74)
3.0 gm., single dose	7M	24, 24, 25, 25, 27, 34, 36	68, 75, 75, 80, 85, 88, 90 (Av. 80)
2.0 gm., then 1.0 gm. every 24 hrs.	2F, 5M	41, 49, 53, 54, 70, 72, 73	50, 55, 55, 59, 72, 74, 80 (Av. 64)
1.0 gm. every 24 hrs.	2F, 1M	62, 67, 70	50, 52, 73 (Av. 58)
1.0 gm., then 0.5 gm. every 12 hrs.	2F, 5M	57, 63, 71, 72, 72, 73, 85	50, 50, 54, 61, 68, 68, 73 (Av. 62)
0.5 gm. every 12 hrs.	1F, 5M	35, 45, 47, 50, 60, 68	59, 60, 64, 73, 75, 82 (Av. 69)
1.0 gm. every 48 hrs.	3F, 4M	17, 27, 36, 62, 62, 65, 66	31, 36, 41, 49, 64, 73, 78 (Av. 53)

Two other dosage regimens were employed for the present study, as indicated in Table 1. In addition, the dose of 1.0 gm. every 24 hours was given to 3 patients without the larger initial dose, so that a total of 7 different dosage regimens were available for comparisons.

In this paper all of the blood levels are reported as concentration in whole blood. Since all or nearly all of the sulfamethoxypyridazine in freshly drawn oxalated human blood was usually found in the plasma and little or none in the blood cells (Nichols and Finland[11] and Nichols, Jones and Finland[12]), these levels in whole blood may be, on the average, only about 60% of the corresponding concentrations found in the plasma.

In the previous study (Nichols and Finland[11]), when doses of 1.0 gm. were given every 48 hours, a slight but definite cumulative effect was noted in the concentrations of sulfamethoxypyridazine in the plasma over a period of several days. This cumulation in the blood was even more striking when doses of 0.5 gm. were given every 12 hours (Jones *et al.*[7]). With the latter dose the blood levels obtained during the first day were rather low. Observations were therefore made with two other dosage regimens in which a larger initial amount was given. The concentrations of drug in whole blood obtained with an initial dose of 2.0 gm. followed by 1.0 gm. every 24 hours are shown graphically in Fig. 1. and those found during treatment with an initial dose of 1.0 gm. followed by 0.5 gm. every 24 hours are depicted in Fig. 2. With both of these regimens there were wide variations in the concentrations found in different patients; these were much greater than could be accounted for by differences in weight of the patients. There was also some cumulative effect in the blood levels of the drug on

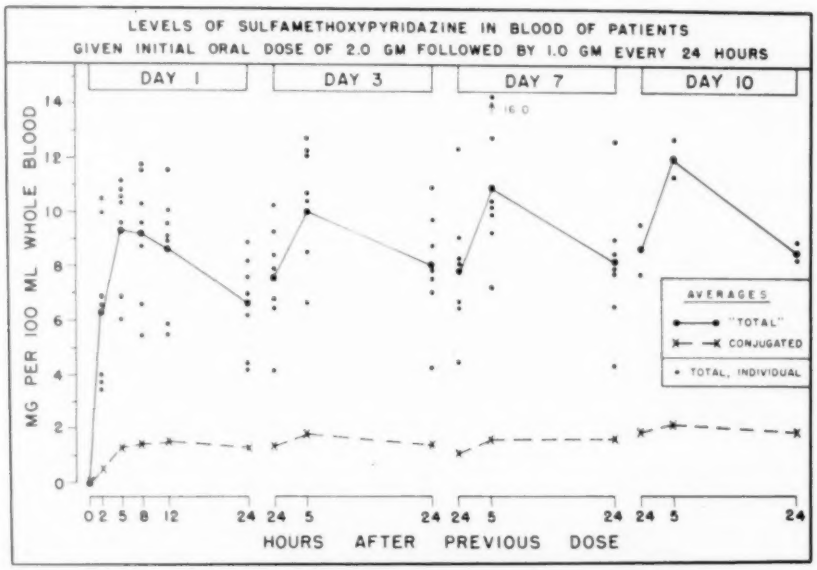

Fig. 1.

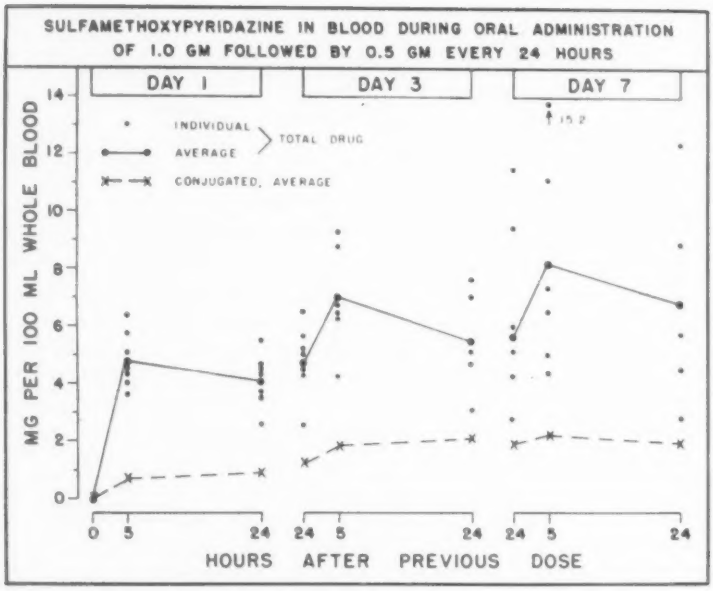
SULFAMETHOXYPYRIDAZINE IN BLOOD DURING ORAL ADMINISTRATION
OF 1.0 GM FOLLOWED BY 0.5 GM EVERY 24 HOURS
DAY 1
DAY 3
DAY 7
INDIVIDUAL
AVERAGE
TOTAL DRUG
CONJUGATED, AVERAGE
15.2
MG PER 100 ML WHOLE BLOOD
0
2
4
6
8
10
12
14
0 5 24 24 5 24 24 5 24
HOURS AFTER PREVIOUS DOSE

Fig. 2.

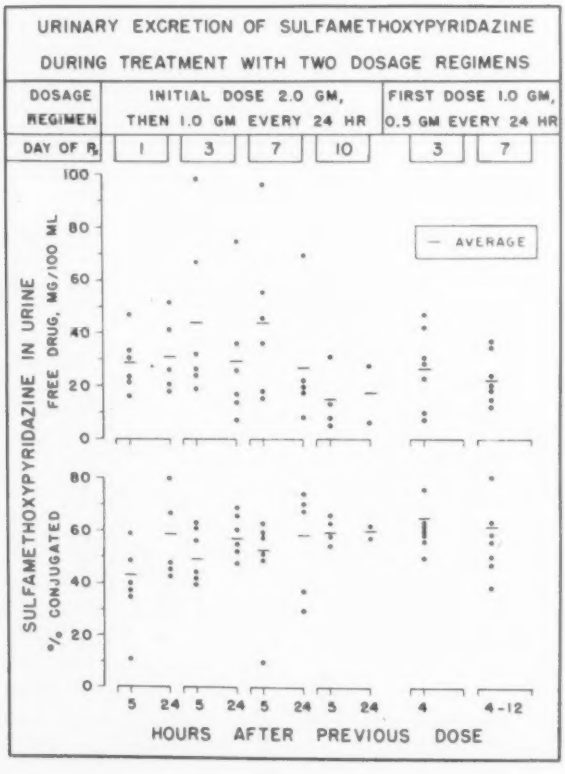
URINARY EXCRETION OF SULFAMETHOXYPYRIDAZINE
DURING TREATMENT WITH TWO DOSAGE REGIMENS
DOSAGE REGIMEN
INITIAL DOSE 2.0 GM, THEN 1.0 GM EVERY 24 HR
FIRST DOSE 1.0 GM, 0.5 GM EVERY 24 HR
DAY OF Rx
1
3
7
10
3
7
AVERAGE
SULFAMETHOXYPYRIDAZINE IN URINE
FREE DRUG, MG/100 ML
% CONJUGATED
0
20
40
60
80
100
5 24 5 24 5 24 5 24 4 4-12
HOURS AFTER PREVIOUS DOSE

Fig. 3.

both regimens, but this was not nearly so marked as in the patients who received 0.5 gm. every 12 hours (Jones *et al.*[7]).

The concentrations of the free drug and the per cent of drug recovered in the conjugated form in the individual specimens of urine are presented in the urine were usually several times those found in the plasma, but concentrations of 50 mg. per 100 ml. or higher of the free drug were not found in any of the urines of the patients on the smaller doses and were infrequent among those who received the larger doses.

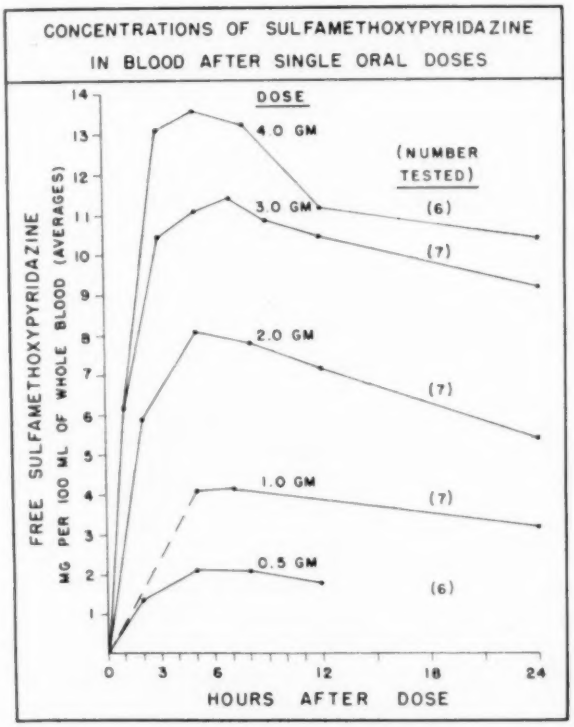

Fig. 4.

graphically in Fig. 3; the average of the values obtained at the various intervals are also shown in this figure. Considering the fact that these were essentially random samples and fluid intake was not controlled and varied markedly in these patients, the variations in concentrations of the drug in the urine, particularly in the average values, were not remarkable. Concentrations of free sulfamethoxypyridazine

Comparisons of the average concentrations of the free drug in whole blood after single or initial doses ranging from 4.0 gm. to 0.5 gm. are shown in Fig. 4. Peak concentrations were reached in each instance within 3 hours and were then sustained over a period of 3 or 4 hours. The average concentrations over most of the first 24 hours appeared to be nearly proportional to the dose, and the decline in

TABLE 2.—BLOOD LEVELS OF SULFAMETHOXYPYRIDAZINE (SMP) AFTER VARIOUS ORAL DOSES

Dosage Regimen	Day of Rx	Hrs. after Previous Dose	Number of Patients	Concentration of SMP in Whole Blood, mg./100 ml.					
				Total			Free		
				High	Low	Average	High	Low	Average
4.0 gm., single dose	–	1	6	11.1	2.3	6.4	9.4	2.5	6.2
		3	6	16.2	11.1	13.5	16.0	10.5	13.1
		5	6	18.0	11.1	15.2	16.0	10.5	13.6
		8	6	18.0	12.6	15.1	16.0	11.1	13.2
		12	6	15.1	11.8	13.1	12.8	10.5	11.2
		24	6	14.5	10.0	12.1	12.8	8.0	10.3
		105	5	5.2	2.0	3.3	4.5	1.3	2.8
3.0 gm., single dose	–	1	7	9.5	4.8	7.5	8.5	4.5	6.2
		3	7	13.8	10.0	11.4	13.0	8.9	10.5
		5	7	15.5	10.5	12.7	12.6	9.8	11.1
		7	7	15.4	12.1	13.5	13.8	10.2	11.4
		9	7	13.5	11.6	12.6	11.9	10.2	10.9
		12	7	12.7	10.0	11.5	11.9	9.0	10.5
		24	7	13.5	8.8	10.6	12.3	7.9	9.2
		48	7	8.5	6.9	7.4	7.2	5.9	6.4
		96	6	4.0	2.7	3.1	3.8	2.3	2.8
		144	6	1.7	1.1	1.3	1.5	0.8	1.1
2.0 gm., then 1.0 gm. every 24 hrs.	1	2	7	10.5	3.7	6.4	9.7	3.4	5.9
		5	7	11.2	6.1	9.4	10.2	5.2	8.1
		8	7	11.7	6.6	9.2	10.9	4.7	7.8
		12	7	11.6	5.5	8.7	9.4	4.6	7.2
		24	7	8.9	4.3	6.7	7.9	3.5	5.4
	3	24	7	10.3	4.2	7.6	9.1	3.5	6.2
		5	7	12.8	6.7	10.1	9.8	5.6	8.3
		24	7	11.0	4.3	8.1	10.0	3.3	6.7
	7	24	7	12.4	4.5	7.9	10.9	3.5	6.8
		5	7	16.0	7.3	10.9	14.0	6.2	9.3
		24	7	12.7	7.4	8.2	11.4	3.4	6.6
	10	24	2	9.6	7.8	8.7	6.9	6.7	6.8
		5	2	12.8	11.4	12.1	10.2	9.6	9.7
		24	2	8.9	8.3	8.6	6.8	6.6	6.7

1.0 gm. every 24 hrs.	1	8	3	5.8	4.3	5.0	4.4	3.5	3.8
		24	3	5.0	3.0	3.8	3.4	2.5	2.9
	3	24	3	7.2	5.2	6.1	4.4	3.6	4.0
		8	3	9.1	8.0	8.5	6.7	6.2	6.5
		24	3	8.2	6.5	7.2	5.7	4.1	5.1
	7	24	3	11.8	7.3	9.0	6.8	4.3	6.0
		8	3	12.9	11.0	11.9	9.4	7.9	8.7
		24	3	13.4	7.6	9.6	8.1	4.9	6.6
1.0 gm., then 0.5 gm. every 24 hrs.	1	5	7	6.4	3.6	4.8	5.7	3.0	4.1
		24	7	5.5	2.6	4.1	3.9	2.6	3.2
	3	24	7	6.5	2.6	4.8	5.1	2.0	3.5
		5	6	9.3	4.3	7.0	6.8	3.5	5.2
		24	5	7.0	3.1	5.5	5.1	1.8	3.4
	7	24	6	11.5	2.8	6.4	6.8	2.7	4.5
		5	6	15.2	4.3	8.2	10.4	3.8	6.0
		24	5	12.4	2.8	6.8	7.9	2.6	4.9
0.5 gm. every 12 hrs.	1	2	5	2.9	0	1.8	2.7	0	1.4
		5	6	2.8	2.2	2.5	2.4	1.3	2.1
		8	5	2.8	1.7	2.4	2.5	1.5	2.1
		12	5	2.3	1.6	2.1	2.0	1.4	1.8
	3	12	6	5.8	4.0	4.9	5.2	3.2	3.9
		5	6	7.4	4.0	6.0	6.8	3.8	5.2
		12	5	6.1	4.1	5.5	5.8	3.9	4.6
	7	12	5	9.2	5.6	7.6	7.4	3.9	6.3
	8	12	5	8.8	6.9	7.7	6.9	4.3	6.0
		5	4	8.4	7.4	7.9	7.3	4.5	6.1
1.0 gm. every 48 hrs.	1	7	7	7.2	3.4	5.1	6.5	2.9	4.3
	2	24	5	6.0	2.1	4.1	4.8	1.6	3.1
	3	48	7	3.8	1.5	2.1	2.3	0.8	1.5
		7	7	8.2	4.6	6.4	7.6	4.0	5.3
	4	24	5	6.6	3.4	4.7	5.0	2.6	3.8
	5	48	7	5.3	1.6	2.7	3.6	1.2	2.0
		7	6	9.6	4.9	7.9	8.3	3.2	6.1
	7	48	6	5.1	1.7	2.9	4.3	1.7	2.2
		7	5	10.6	4.6	7.6	9.2	3.4	6.3
	9	48	5	6.7	1.7	3.8	4.9	1.6	2.5
		7	4	10.9	5.2	7.6	9.6	4.3	5.9
	11	48	4	4.8	1.7	3.2	2.9	1.5	2.2
		7	4	10.7	4.9	7.8	9.5	4.1	6.2
	13	48	4	4.2	2.2	3.7	3.5	1.4	2.3
		7	3	11.2	5.5	8.3	9.1	4.4	6.7
	15	48	5	3.8	2.9	3.3	2.9	2.1	2.4
	15–19	7	8	11.2	6.1	8.0	9.1	4.4	6.4
	17–21	48	6	4.8	2.6	3.7	3.8	1.8	2.8

the levels was roughly at the same rate, although that appears to be less certain from these data.

The maximum, minimum and average values for the concentrations of both free and total sulfamethoxypyridazine in whole blood at about the time of the expected peaks, and the lowest values obtained just prior to some of the doses during continuous therapy are listed in Table 2; additional values for the first and other early doses are also included in this table. Only moderate variations, usually within a 2-fold range, were noted in the concentrations achieved by different individuals at the same time following the same doses, although greater variations were observed after several days of treatment with doses of 0.5 gm. every 24 hours. Here again, the average level at any given time during treatment was roughly proportional to the dose.

Table 3 lists the ranges and average values for the concentrations of free sulfamethoxypyridazine in the urines collected during administration of the various dosages; the per cent of the corresponding values for total drug that was determined as the conjugated form are also shown in this table. As already noted, fluid intake was not controlled in these cases, and nearly one-half of the specimens were collected 24 hours or longer after the preceding doses. There were, therefore, wide variations in the concentrations of urine obtained at the same time from different individuals on the same dosage regimen. Also, as might be expected from the well sustained blood levels of free drug associated with its low renal clearance, as compared with the low concentrations of conjugated drug in the blood accompanying the much faster rate of clearance of the latter, the concentrations of free drug in the urine were relatively low, whereas the proportion of the urinary sulfonamide determined as the conjugated form was relatively large when compared with other, more rapidly excreted sulfonamides. This was well illustrated in the comparisons with the concentrations of drug excreted after doses of the closely related sulfachloropyridazine (Jones and Finland[6], and Jones *et al.*[7]).

Comment. From the data presented and from other similar observations it should be possible to select the optimum dosage regimen for the various purposes for which this sulfonamide may prove useful. Obviously only broad deductions can be drawn from these data, since the number of subjects studied with each regimen was small and the individual variations were often too large to permit detailed statistical verification of the significance of all the differences observed. Statistical analyses are therefore omitted. At least some of the wide variations observed in the values obtained in different subjects may be accounted for by differences in weight of the patients, and particularly in their ages, for although gross impairment of renal function was not observed in any of them, there was undoubtedly some reduction in this function in the elderly patients.

From the data shown in Figs. 1 and 4 and in Table 2 it would seem that a single or initial oral dose of 2.0 gm. of sulfamethoxypyridazine should provide, within 3 to 5 hours, and maintain over the rest of a 24-hour period, concentrations of free sulfonamide in whole blood that may be considered optimum when compared with those obtained with considerably larger doses of other active sulfonamides. This is particularly true when one takes into consideration the concentrations in the plasma, which although not shown here were higher than the corresponding levels in whole blood by from 50% to nearly

100% (with hematocrits ranging from 35 to 50%).

The optimum levels of this or other sulfonamides that should be maintained in blood or plasma cannot be stated categorically, for that will vary with the purpose for which the drug is used. It is reasonable to assume, from other available data, that the incidence and severity of untoward reactions such as fever, rashes and perhaps also bone marrow depression, may be in proportion to the concentrations of the drugs that are maintained in the blood and tissues (Lehr[8]). It would, therefore, be necessary to weigh the therapeutic and prophylactic potentialities of the various dosages and the duration of their use against their potentialities for producing untoward effects, taking into consideration the likelihood that the latter would probably affect only a relatively small proportion of the total number of patients treated.

From these considerations, and until more, well documented information becomes available from patients actually treated under controlled conditions, it seems justifiable to make some recommendations as to proper dosage regimens. If a short course of treatment is to be given for a serious infection or if the drug is to be used for the treatment of infections due to organisms that are not highly sensitive, it would be reasonable to recommend for adults an initial dose of 2.0 gm. followed by 1.0 gm. every 24 hours for a few days (up to one week) after which the drug should be discontinued or reduced to 0.5 gm. daily. For most therapeutic purposes, however an initial dose of 1.0 gm. followed by 0.5 gm. every 24 hours should be adequate, although the larger initial dose may be desirable to achieve and maintain higher levels, especially during the first day and even for several days.

It is desirable, when daily doses are given even in amounts of only 0.5 gm. each 24 hours to check the blood levels after a few days in order to ascertain that absorption is adequate and that there is no undue retention of the drug. This should be mandatory for this drug, as with any other sulfonamide, when used in patients who are suspected of having impaired renal function, if treatment is to be continued with safety. Blood counts should also be done during prolonged treatment.

For prophylactic purposes the dose of 0.5 gm. daily is probably slightly excessive, but may be necessary as a suppressive dose in patients with urinary tract infections due to susceptible organisms (Grieble and Jackson[5], and Jones and Finland[6]). Smaller or less frequent doses are clearly indicated in the prevention of streptococcal infections and rheumatic fever. Lepper, Simon and Marienfeld[9] found a dose of 30 mg. per kg. once a week to be successful in their preliminary observations. This would correspond to a dose of 1.5 to 2.0 gm. once weekly for adolescents or adults weighing 50 to 67 kg., respectively, or a dose of 0.5 to 1.0 gm. in children weighing up to 33 kg. Grieble and Jackson[5] using 0.5 gm. daily over periods of 1 to 150 days in patients with urinary tract infections reported mean concentrations of free sulfonamide in blood, serum and urine to be 9.0, 10.3 and 17.1 mg. per 100 ml., respectively; the corresponding concentrations of total sulfonamide were 9.7, 12.1 and 28.9. These figures for blood and serum, incidentally, suggest a greater penetration of this drug into red blood cells than was demonstrated in freshly separated specimens of oxalated blood after single doses in humans in this laboratory, and correspond more closely to the findings reported by Roepke, Maren and Mayer[13] in dogs.

Summary and Conclusions. Concentrations of sulfamethoxypyridazine

TABLE 3.—CONCENTRATIONS OF SULFAMETHOXYPYRIDAZINE IN URINE AFTER VARIOUS ORAL DOSES

Dosage Regimen	Day of Rx	Hrs. after Previous Dose	Number of Patients	Sulfamethoxypyridazine (SMP) in Urine					
				Free SMP, mg./100 ml.			% Conjugated		
				High	Low	Average	High	Low	Average
4.0 gm., single dose	–	1	5	21	0	7	56	32	41
		3	5	38	19	26	52	21	35
		5	5	68	41	50	59	26	43
		8	5	71	38	49	65	38	48
		12	5	68	31	47	63	34	49
		24	5	53	14	35	79	46	58
		48	5	50	18	30	77	41	54
		105	5	12	6	8	67	42	53
3.0 gm., single dose	–	1	7	15	1.3	5.4	32	0	21
		3	7	40	0.9	22	42	24	31
		5	7	48	6.	29	48	23	37
		7	7	65	3.1	33	55	33	44
		12	7	68	6	34	64	38	51
		24	7	40	14	28	67	37	51
		48	7	25	8	18	66	32	47
		96	6	10	4.1	8	74	53	68
		144	6	4.1	1.5	3.3	77	54	66
2.0 gm., then 1.0 gm. every 24 hrs.	1	5	6	47	16	29	59	11	43
		24	5	52	18	31	80	43	59
	3	5	5	99	9	44	63	40	49
		24	6	75	5	29	69	48	57
	7	5	6	96	16	44	69	10	52
		24	5	70	8	27	74	30	58
	10	5	4	31	5	14	66	54	57
		24	2	28	6	17	62	58	60

1.0 gm. every 24 hrs.	4	6	3	64	13	37	60	32	47
	7	24	3	63	35	38	67	48	56
1.0 gm., then 0.5 gm. every 24hrs. .	3	4	7	47	7	26	76	50	60
	7	4, 12	7	35	13	23	81	39	63
0.5 gm. every 12 hrs.	1	12	5	21	3.9	13	85	49	63
	3	5	6	50	4.3	25	84	52	66
	6	6	4	50	25	37	86	55	67
	8	6	4	57	29	41	67	54	60
1.0 gm. every 48 hrs.	1	7	7	26	2.6	14	73	26	53
	2	24	6	19	9	20	81	36	62
	3	48	6	14	2.3	9	87	47	64
	3	7	7	36	2.3	19	87	47	63
	4	24	5	33	13	21	67	47	56
	5	48	6	30	3.1	14	76	52	63
	5	7	5	35	7.0	25	78	42	60
	7	48	5	24	5.5	11	72	57	66
	7	7	5	37	17	27	74	49	59
	9	48	5	14	9.0	10	87	59	72
	9	7	4	23	6.8	16	85	50	68
	11	48	3	9	3.3	28	78	53	68
	11–19	7	7	46	3.3	21	94	55	68
	13–21	48	7	31	5.3	16	86	54	67

(Kynex) in blood and urine were determined in normal men after single oral doses of 4.0 and 3.0 gm. and in hospitalized adult patients under treatment with 5 different dosage regimens. The data are offered as an aid in the selection of proper dosage regimens for the prophylactic and therapeutic application of this new and potentially useful sulfonamide.

In the treatment of most susceptible infections in adults an initial dose of 1.0 gm. followed by 0.5 gm. every 24 hours appears to provide adequate concentrations in the blood of most patients. An initial dose of 2.0 gm. is suggested when higher blood levels are desired early in the course of treatment, and daily doses of 1.0 gm. may be required during the first few days when high and well sustained levels are required for infections that are severe or for those caused by less susceptible organisms. For prophylaxis of streptococcal infections and rheumatic fever the dose of 30 mg. per kg. of body weight (1.5 to 2.0 gm. for adolescents or adults weighing up to 67 kg.) once a week, as suggested by Lepper, Simon and Marienfeld[9], appears to be reasonable and adequate.

When treatment with sulfamethoxypyridazine is continued for a week or longer, it is highly desirable to determine the concentration of the drug in the blood in order to ascertain whether absorption is adequate without excessive cumulation; this is mandatory when this drug (or any other sulfonamide) is used in patients known or suspected of having impaired renal function.

REFERENCES

1. **Boger, W. P., Strickland, C. S.,** and **Gyle, J. M.:** Antibiot. Med. & Clin. Ther., **3**, 378, 1956.
2. **Bratton, A. C.,** and **Marshall, E. K.:** Proc. Soc. Exper. Biol. & Med., **92**, 637, 1956.
3. **Foerster, D. K., Martin, W. J., McGuckin, W. F.,** and **Nichols, D. R.:** Proc. Staff Meet., Mayo Clin., **31**, 678, 1956.
4. **Frisk, A. R.,** and **Wassen, A.:** Antibiotic Annual 1956–1957, p. 424.
5. **Jackson, G. G.,** and **Grieble, H. G.:** Ann. New York Acad. Sci., **69**, 493, 1957.
6. **Jones, W. F., Jr.,** and **Finland, M.:** Ann. New York Acad. Sci., **69**, 473, 1957.
7. **Jones, W. F., Jr., Ziai, M., Cherrick, G. R.,** and **Finland, M.:** Proc. Soc. Exper. Biol. & Med., in press.
8. **Lehr, D.:** Ann. New York Acad. Sci., **69**, 485, 1957.
9. **Lepper, M. H., Simon, A. J.,** and **Marienfeld, C. J.:** Ann. New York Acad. Sci., in press.
10. **Litchfield, J. T.:** Tr. XX International Phys. Cong., Brussels, 1956.
11. **Nichols, R. L.,** and **Finland, M.:** J. Lab. & Clin. Med., **49**, 410, 1957.
12. **Nichols, R. L., Jones, W. F., Jr.,** and **Finland, M.:** Proc. Soc. Exper. Biol. & Med., **92**, 637, 1956.
13. **Roepke, R. R., Maren, T. H.,** and **Mayer, E.:** Ann. New York Acad. Sci., **69**, 457, 1957.
14. **Ross, S., Ahrens, E.,** and **Zaremba, E.:** Ann. New York Acad. Sci., **69**, 501, 1957.
15. **Walker, W. F.,** and **Hamburger, M.:** Ann. New York Acad. Sci., **69**, 509, 1957.

(Interlingua on page 606)

IRON ENZYMES IN IRON DEFICIENCY. I. CYTOCHROME C*

By Ernest Beutler, M.D.

(From The Argonne Cancer Research Hospital and The Department of Medicine, University of Chicago, Chicago, Illinois)

Iron deficiency anemia is among the most common of clinical disorders. Yet, the symptoms of iron deficiency anemia, the fatigue, headache, dyspnea and paresthesias, have received little attention. While it is generally assumed that these symptoms are due chiefly to the anemia, no experimental evidence supports this view.

It is understandable that attention should be focused on the decreased hemoglobin level of these patients because hemoglobin is the most easily measured iron compound in the body. Furthermore, it is often believed no other origin for the symptoms of iron deficiency is possible, because of the frequently repeated statement that the body tenaciously guards its iron enzymes in iron deficiency (Goodman and Gilman[9], Hahn[12], Paul[20], and Theorell *et al.*[23]). By exclusion, this leaves only the hemoglobin for consideration. As attractive as the view that iron enzymes are normal in iron deficiency may be, however, a search for evidence is less rewarding. Indeed, the evidence appears to be limited to the study by Hahn and Whipple[13] of iron in the perfused tissues of a single, iron-deficient dog. Even though these authors exercised considerable care to free the tissue from blood, it is apparent, when we consider that the cytochrome c iron of a gram of muscle is equivalent to the amount of iron contained in one-sixth thousandth ml. of blood, that it is impossible to draw conclusions about enzyme iron from iron in the ash of perfused tissues.

A number of clinical observations are not explained satisfactorily by assuming that iron deficiency is a disorder in which the effect of the blood is of the greatest importance. It has been observed frequently that iron-deficient patients receiving iron therapy, particularly when given parenterally, undergo marked symptomatic improvement before any rise in hemoglobin occurs (Beutler[3], Brown *et al.*[7], Heilmeyer and Plötner[14], and Jennison and Ellis[17]). Furthermore, we have been impressed by the almost complete lack of correlation between the degree of anemia and the severity of symptoms observed in our iron-deficient patients[3]. The epithelial signs that occur in iron deficiency, the changes in nails, mouth and esophagus, cannot be explained on the basis of the anemia alone and, indeed, Waldenström has claimed that they may occur in the absence of any anemia[25].

In view of these findings, it is surprising that more exhaustive studies of iron enzymes in iron deficiency have not been carried out. A few isolated pertinent observations have been made, usually secondary to investigations of copper deficiency. Thus, Cohen and Elvehjem[8] found that the absorption bands of cytochromes b and c were reduced in intensity in iron- and copper-deficient rats and were not restored by treatment with copper alone. Indophenol oxidase (cytochrome oxidase) and cytochrome a, on the other hand,

*Presented before the American Society for Clinical Investigation at Atlantic City, N.J., May 6, 1957.

were reduced markedly in copper and iron deficiency and were restored to normal by the addition of copper alone. Vannotti[24] refers to lower cytochrome c levels in iron-deficient rats but presents no data to support this contention. Most recently, Gubler, Cartwright, and Wintrobe[10] determined the cytochrome c and cytochrome oxidase content of tissues of 3 pigs who received a milk diet supplemented with copper. There was some reduction in cytochrome c concentration but no reduction in total organ cytochrome c of these iron-deficient animals. No change in cytochrome oxidase was observed in these pigs but copper-deficient pigs showed a marked decrease in this enzyme. These authors attempted to explain the differences between their findings and the conclusions of Hahn and Whipple[13] on the basis of a difference in the severity of the iron deficiency of their animals. The catalase content of iron-deficient rat livers was found to be essentially normal by Adams[1], but Schultze and Kuiken[22] found decreased catalase activity in the liver, kidney, and blood of iron-deficient rats. Lahey *et al.*[18] reported a slight decrease in liver catalase of iron-deficient pigs, but no change in red cell or kidney catalase. Observations on the effects of iron deficiency on myoglobin have also yielded contradictory results. Early necropsy observations appear to show some decrease in myoglobin in the muscles of iron-deficient patients (Gunther[11]). These reports are difficult to evaluate. In some of their earlier studies, Whipple, Groth and Robscheit-Robbins concluded that there was some decrease in the myoglobin content of muscles of dogs made anemic by bleeding or by being fed a bread diet[26,27]. Subsequently, however, Hahn and Whipple concluded that the anemia of bleeding caused no decrease in myoglobin[13]. The method used in these studies was not specific for myoglobin and in some instances it is not certain that the animals were actually iron deficient. In Biörck's[6] careful studies of myoglobin in man, no clear distinction was made between types of anemia. His data suggest, however, that cases 72 and 127 were iron deficient. In these patients there was no decrease in myoglobin concentration of the muscles examined. Gubler, Cartwright and Wintrobe[10], on the other hand, reported lowered myoglobin levels in the muscles of 3 iron-deficient pigs.

A careful re-examination of iron enzymes in iron deficiency, therefore, appeared to be in order. As a model we chose to measure the concentration of cytochrome c because of its well-known biologic importance and because its concentration may be measured spectrophotometrically entirely independently of any blood that may be retained in the tissues.

Methods and Materials. Fifty to 70 gm. Sprague-Dawley rats, aged 4 weeks, were offered either a normal diet or iron-deficient diet. The normal diet consisted of a modification of the 4c diet used by Wissler and his co-workers[28]. The iron-deficient diet was identical except that no ferrous sulphate was included. The iron content of the normal diet was 21 to 22 mg. of iron per 100 gm., while the iron-deficient diet contained 1 to 2 mg. of iron per 100 gm. At appropriate intervals all rats were bled by cardiac puncture. Hemoglobin concentrations were determined spectrophotometrically as alkaline oxyhemoglobin using individually calibrated pipettes. Red cell counts were performed using National Bureau of Standard certified pipettes, and hematocrits were measured by means of micro-hematocrit tubes.

The concentration of cytochrome c in the liver and kidneys was determined using a modification* of the method of Potter and DuBois[21].

*The entire procedure was carried out in the cold. The precipitate formed at pH 7.0, was re-extracted at pH 3.5. Centrifugation time in the final trichloracetic acid concentration step was increased to one hour and was carried out in a cooled centrifuge.

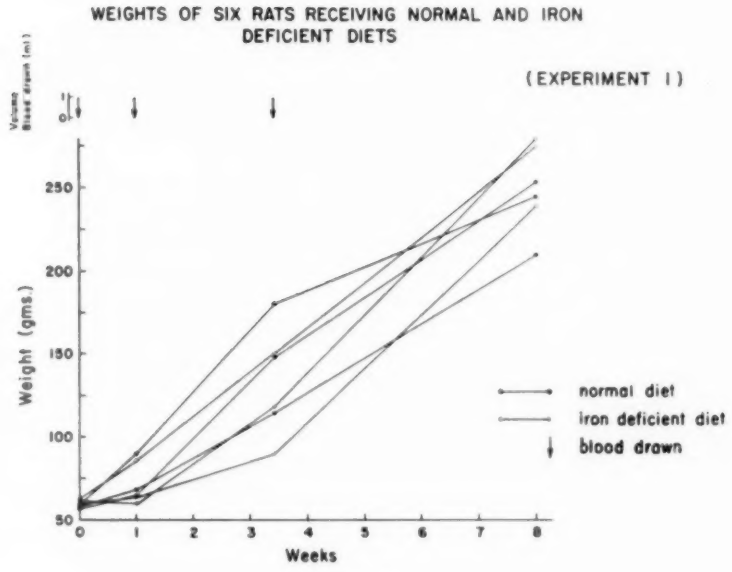
WEIGHTS OF SIX RATS RECEIVING NORMAL AND IRON DEFICIENT DIETS
(EXPERIMENT I)
Volume Blood drawn (ml)
Weight (gms.)
250
200
150
100
50
0 1 2 3 4 5 6 7 8
Weeks
normal diet
iron deficient diet
blood drawn

Fig. 1.

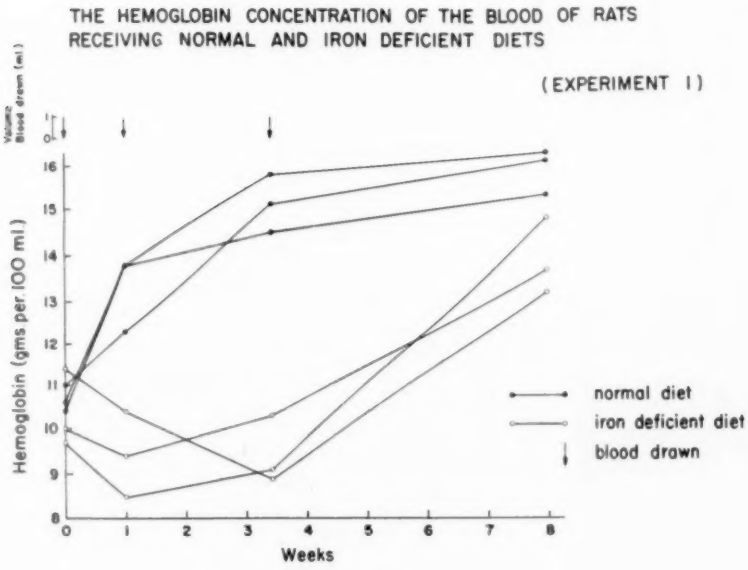
THE HEMOGLOBIN CONCENTRATION OF THE BLOOD OF RATS RECEIVING NORMAL AND IRON DEFICIENT DIETS
(EXPERIMENT I)
Volume Blood drawn (ml)
Hemoglobin (gms per 100 ml)
16
15
14
13
12
11
10
9
8
0 1 2 3 4 5 6 7 8
Weeks
normal diet
iron deficient diet
blood drawn

Fig. 2.

Experimental and Results. One group of 3 rats was fed the normal diet and another group of 3 rats, the iron-deficient diet. One milliliter of blood was drawn by heart puncture at the initiation of the experiment, after 1 week, and after 3½ weeks. All of the animals gained weight normally (Fig. 1) and remained in good general health. However, the hemoglobin content of the blood of the rats receiving the iron-deficient diet fell below that of rats receiving the normal diet (Fig. 2). At the end of 8 weeks, the rats

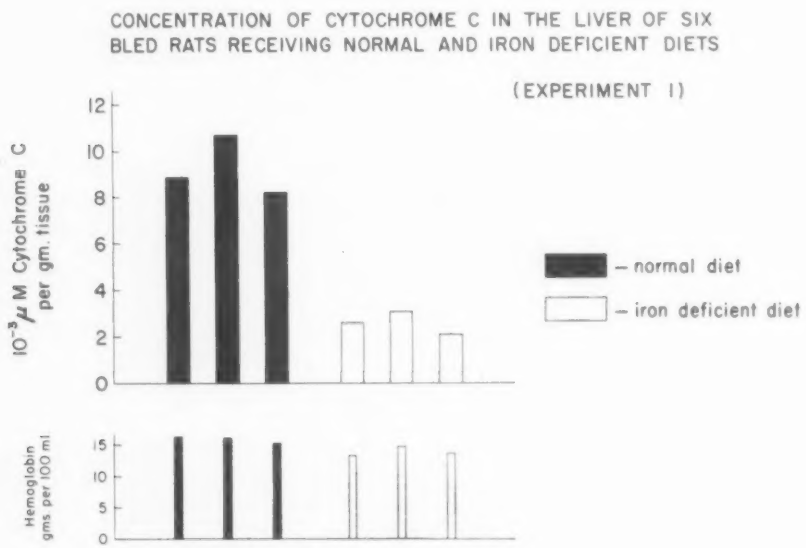

Fig. 3.

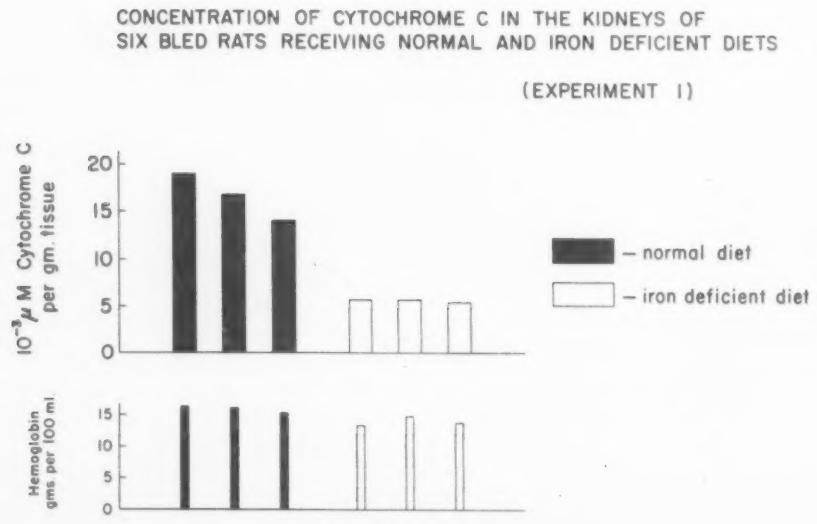

Fig. 4.

were sacrificed and the cytochrome c concentration of liver and kidney was determined (Figs. 3 and 4). In spite of the minimal decrease of the hemoglobin concentration of the blood of the iron-deficient rats, a marked decrease in cytochrome c concentration was observed.

The second experiment was very similar to the first. Bleeding was carried out periodically, and the animals

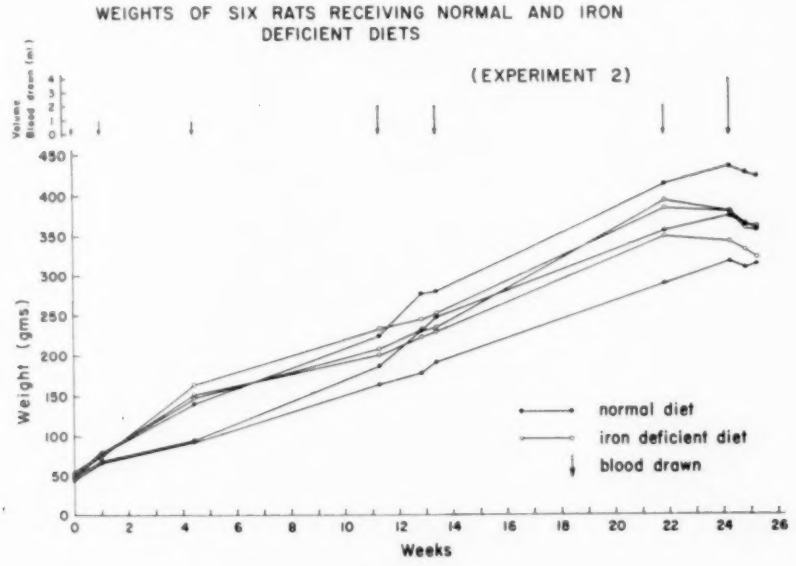

Fig. 5.

receiving the iron-deficient diet became anemic (Figs. 5 and 6). The rate of bleeding was reduced so that the hemoglobin returned to normal even in the iron-deficient animals. At the end of approximately 26 weeks, the rats were sacrificed and peripheral blood counts and liver and kidney cytochrome c determinations were carried out. The pertinent data are presented in Table 1 and Figs. 7 to 10. While the decrease in concentration of kidney cytochrome was slight, the decrease in the concentration of liver cytochrome was striking. In the first experiment the kidney and liver weights were not recorded. In this experiment such measurements were made, and it was, therefore, possible to express the kidney and liver cytochrome c as the total amount of cytochrome c in these organs. Because the liver and kidneys of the iron-deficient animals were somewhat smaller than those of the control animals, this served to accentuate the difference (Figs. 9 and 10).

Discussion. Cytochrome c was chosen only as an example of an important iron enzyme. We have no reason to suggest that cytochrome c is particularly important in the symptomatology of iron deficiency, or even that such marked degrees of cytochrome c depletion as were observed result in any functional disturbance. However, these experiments do show conclusively that all iron enzymes are

TABLE 1.—FINDINGS IN 6 PERIODICALLY BLED RATS RECEIVING NORMAL OR IRON-DEFICIENT DIET FOR 26 WEEKS

(Experiment 2)

							Liver cytochrome c			Kidney cytochrome c	
Rat	*Diet*	*Wt. gm.*	*Hb. gm. per 100 ml.*	*RBC $10^6/mm^3$*	*Hct. %*	*Liver Wt. gm.*	*concentration 10^{-3} μM/gm.*	*total 10^{-3} μM*	*Kidney Wt. gm.*	*concentration 10^{-3} μM/gm.*	*total 10^{-3} μM*
A	Normal	315	14.6	6.02	44	11.6	7.8	91	2.67	25.2	67.3
B	Normal	360	14.7	6.75	47	11.9	8.7	104	2.50	24.3	60.8
C	Normal	424	15.8	6.70	48	11.3	10.6	120	2.82	23.2	65.4
D	Iron-Def.	362	15.2	7.16	47	8.3	2.8	23	2.19	17.7	38.8
E	Iron-Def.	323	14.6	7.37	44	6.6	1.7	11	1.73	16.3	28.2
F	Iron-Def.	357	14.1	7.15	43	9.8	2.0	20	2.63	21.3	56.0

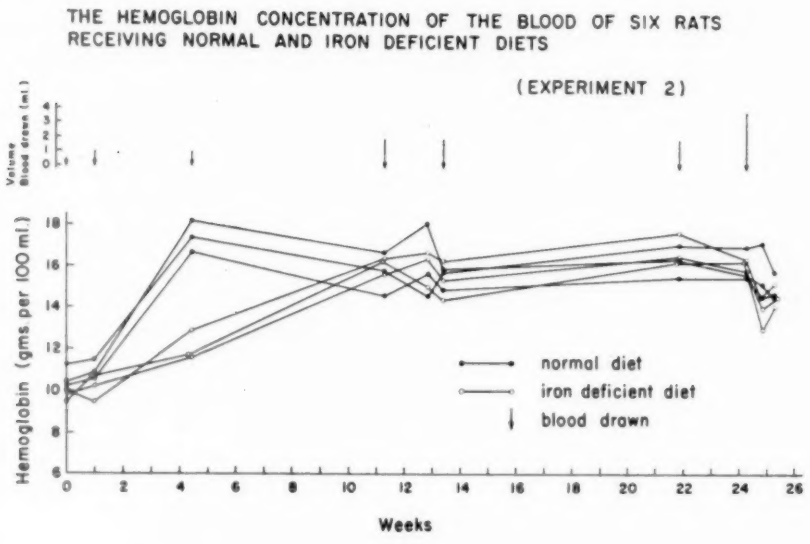
THE HEMOGLOBIN CONCENTRATION OF THE BLOOD OF SIX RATS
RECEIVING NORMAL AND IRON DEFICIENT DIETS
(EXPERIMENT 2)
Volume Blood drawn (ml.)
Hemoglobin (gms per 100 ml.)
normal diet
iron deficient diet
blood drawn
Weeks

Fig. 6.

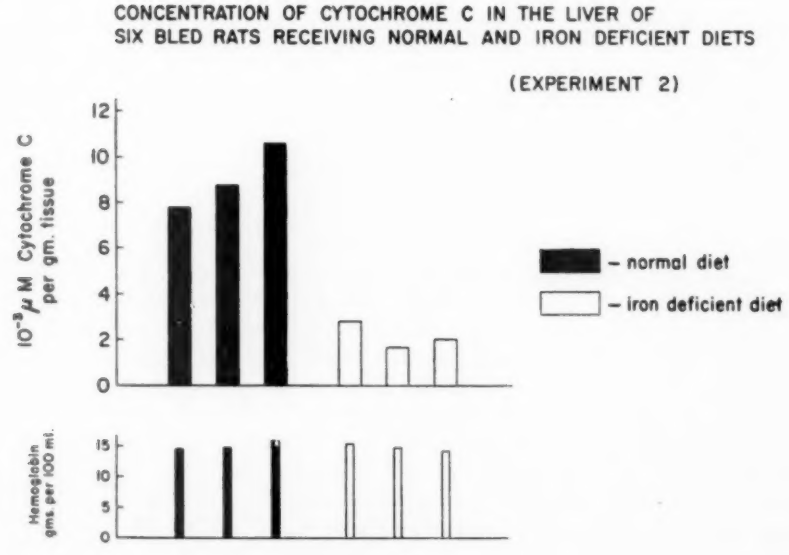
CONCENTRATION OF CYTOCHROME C IN THE LIVER OF
SIX BLED RATS RECEIVING NORMAL AND IRON DEFICIENT DIETS
(EXPERIMENT 2)
$10^{-3}\mu$ M Cytochrome C per gm. tissue
– normal diet
– iron deficient diet
Hemoglobin gms. per 100 ml.

Fig. 7.

not tenaciously protected by the iron-deficient animal. On the contrary, the enzyme that we have examined in this study, cytochrome c, is more susceptible to depletion than is hemoglobin. Available evidence indicates that the turnover of cytochrome c is quite slow (Helwig and Greenberg[15], and Theorell *et al.*[23]). It would seem that when only a small amount of iron is available, the hemoglobin synthesizing mechanism is more successful in competing for it than is the cytochrome c synthesizing mechanism. In view of these findings, the concept that the symptomatology of iron deficiency is related to disturbances in tissue metabolism rather than to mere reduction in the circulating hemoglobin seems a not unreasonable one. In this respect, an analogy between the symptoms of pernicious anemia and those of iron deficiency seems germane.

The finding that cytochrome c may be depleted in iron-deficient animals without anemia allows us to speculate whether the iron-deficient state may be symptomatic in patients without anemia. That this may be the case has been suggested previously by Jasinski and Roth[16]. The extensive work that these authors have done on this subject has achieved little acceptance, however. This is probably due to the fact that their claims of symptomatic benefit in patients given iron are based on studies in which there were no controls. Furthermore, evidence that the non-

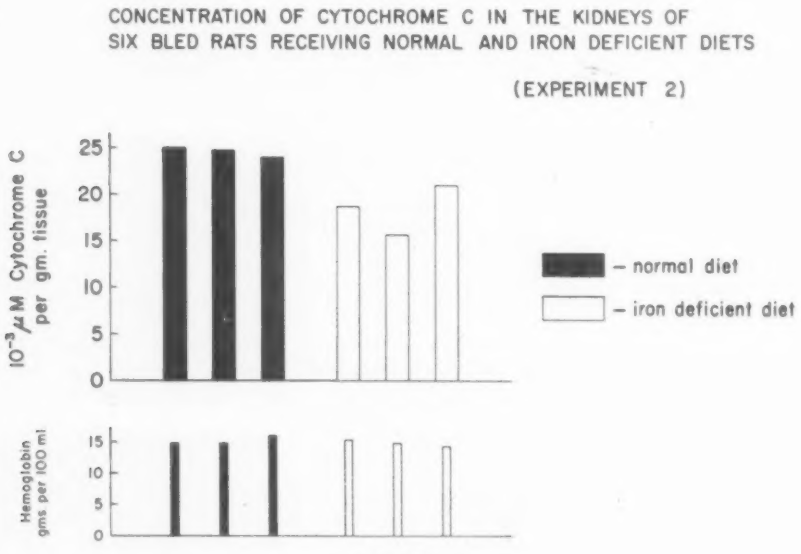

Fig. 8.

anemic patients which they have treated are really iron-deficient is based on oral iron tolerance curves and is, therefore, unacceptable (Beutler, Robson and Buttenwieser[2,5]). Although Jasinski and Roth proposed that symptoms in nonanemic iron deficiency were caused by depletion of iron, they presented no evidence that depletion of iron enzymes actually occurs in iron-deficient patients.

Because of the well-known precarious state of iron balance of menstruating women (Moore and Dubach[19]),

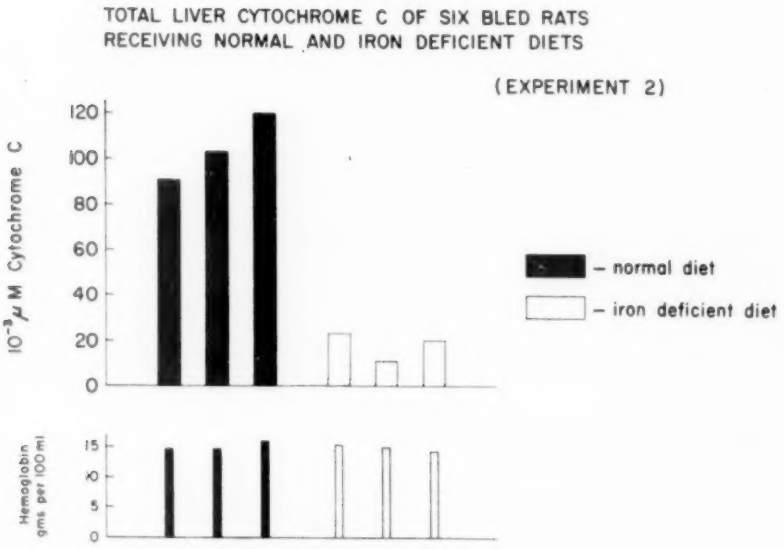
TOTAL LIVER CYTOCHROME C OF SIX BLED RATS
RECEIVING NORMAL AND IRON DEFICIENT DIETS
(EXPERIMENT 2)
$10^{-3}\mu$ M Cytochrome C
120
100
80
60
40
20
0
– normal diet
– iron deficient diet
Hemoglobin gms per 100 ml
15
10
5
0

Fig. 9.

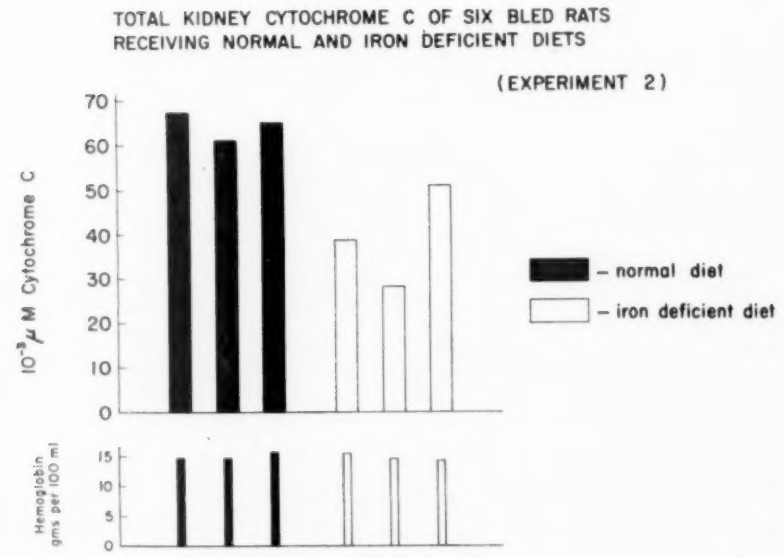
TOTAL KIDNEY CYTOCHROME C OF SIX BLED RATS
RECEIVING NORMAL AND IRON DEFICIENT DIETS
(EXPERIMENT 2)
$10^{-3}\mu$ M Cytochrome C
70
60
50
40
30
20
10
0
– normal diet
– iron deficient diet
Hemoglobin gms per 100 ml
15
10
5
0

Fig. 10.

however, the possibility that iron deficiency without anemia may give rise to symptoms seems to be a very exciting one. It is interesting in this respect that we have recently been able to demonstrate that a surprisingly high proportion of abnormally tired women have depleted bone marrow iron stores although their circulating hemoglobin is normal (Beutler, Robson and Buttenwieser[5]). We are now investigating the responsiveness of the symptoms of such patients to iron therapy, using the double-blind technique[4]. At present, one can only speculate regarding the relationship between nonanemic iron depletion and symptoms, but it is clear that in iron deficiency, not only the blood but also the tissues deserve consideration.

Summary. It is frequently assumed that the symptoms of iron deficiency are due to the decrease in the circulating hemoglobin, but no evidence supports this view. The concept that iron enzymes are tenaciously guarded by the body in iron deficiency has been expressed frequently but surprisingly little work has been done to evaluate the state of iron enzymes in iron deficiency. In this study, cytochrome c has been measured by a highly specific spectrophotometric method which depends upon enzymatic oxidation and reduction of this enzyme. Rats were fed either a normal or an iron deficient diet and were then bled at appropriate intervals. A marked decrease in the cytochrome c content of the liver and kidneys of iron-deficient rats was demonstrated when compared with the control group. This decrease greatly exceeded the slight decrease in hemoglobin concentration. Although no conclusions may be drawn from these studies about the role of cytochrome c in the symptoms of iron-deficiency anemia, it is demonstrated conclusively that all iron enzymes are not "inviolate," as was claimed by Hahn and Whipple. This finding lends some experimental support to the concept that the symptomatology of iron deficiency may be related to disturbances of tissue metabolism rather than to a mere reduction in the circulating hemoglobin. It is also possible that iron depletion without anemia may give rise to symptoms, but this important possibility remains unproved.

ACKNOWLEDGMENT: The author gratefully acknowledges the helpful advice of Dr. Eugene Goldwasser and the technical assistance of Miss Ellen Buttenweiser and Mr. Embe Alexander.

REFERENCES

1. **Adams, D. H.:** Biochem. J., **54**, 328, 1953.
2. **Beutler, E.:** New England J. Med., **256**, 692, 1957.
3. **Beutler, E.:** Unpublished observations.
4. **Beutler, E.,** and **Gurney, C.:** Unpublished observations.
5. **Beutler, E., Robson, M.,** and **Buttenwieser, E.:** Ann. Int. Med., in press.
6. **Biörck, G.:** Acta med. Scandinav., **226**, 7, 1949.
7. **Brown, E. B., Moore, C. V., Reynafarje, C.,** and **Smith, D. E.:** J. Am. Med. Assn., **144**, 1084, 1950.
8. **Cohen, E.,** and **Elvehjem, C. A.:** J. Biol. Chem., **107**, 97, 1934.
9. **Goodman, L. S.,** and **Gilman, A.:** The Pharmacologic Basis of Therapeutics, 2nd ed., New York: The Macmillan Co., 1955.
10. **Gubler, C. J., Cartwright, G. E.,** and **Wintrobe, M. M.:** J. Biol. Chem., **224**, 533, 1957.
11. **Gunther, H.:** Virchows Arch., **230**, 146, 1921.
12. **Hahn, P. F.:** Medicine, **16**, 249, 1937.
13. **Hahn, P. F.,** and **Whipple, G. H.:** Am. J. Med. Sci., **191**, 24, 1936.
14. **Heilmeyer, L.,** and **Plötner, K.:** Das Serumeisen und die Eisenmangelkrankheit (Pathogenese, Symptomatologie und Therapie). Jena: Verlag von Gustav Fischer, 1937.

15. **Helwig, H. L.,** and **Greenberg, D. M.:** J. Biol. Chem., **198,** 703, 1952.
16. **Jasinski, B.,** and **Roth, O.:** Larvierte Eisenmangelkrankheit. Basel: Benno Schwabe & Co., 1954.
17. **Jennison, R. F.,** and **Ellis, H. R.:** Lancet, **2,** 1245, 1954.
18. **Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E.,** and **Wintrobe, M. M.:** Blood, **7,** 1053, 1952.
19. **Moore, C. V.,** and **Dubach, R.:** J. Am. Med. Assn., **162,** 197, 1956.
20. **Paul, K.-G.:** The Iron-Containing Enzymes. A. Cytochromes. *In* The Enzymes By **James B. Sumner** and **Karl Myrback,** Vol. II, Part 1, p. 357. New York: Academic Press, 1951.
21. **Potter, V. R.,** and **DuBois, K. P.:** J. Biol. Chem., **142,** 417, 1942.
22. **Schultze, M. O.,** and **Kuiken, K. A.:** Ibid., **137,** 727, 1941.
23. **Theorell, H., Béznak, M., Bonnichsen, R., Paul, K.-G.,** and **Akeson, A.:** Acta chem. Scand., **5,** 445, 1951.
24. **Vannotti, A.:** Schweiz. med. Wchnschr., **79,** 261, 1949.
25. **Waldenström, J.:** Acta med. Scandinav. Suppl. **90,** 380, 1938.
26. **Whipple, G. H.:** Am. J. Physiol., **76,** 708, 1926.
27. **Whipple, G. H., Groth, A. H.,** and **Robscheit-Robbins, F. S.:** Ibid., **87,** 185, 1928.
28. **Wissler, R. W., Woolridge, R. L., Steffee, C. H., Jr.,** and **Cannon, P. R.:** J. Immunol., **52,** 267, 1946.

SUMMARIO IN INTERLINGUA

Enzymas Ferrifere in Carentia de Ferro. I. Cytochromo C

Il es frequentemente asserite que le symptomas de carentia de ferro resulta de un reduction del hemoglobina circulante, sed iste conception ha nulle supporto objective. Le notion que le enzymas ferrifere es tenacemente preservate per le corpore que suffre de carentia de ferro ha essite exprimite frequentemente, sed surprendentemente pauc investigationes ha essite executate pro evalutar le stato del enzymas ferrifere in carentia de ferro. In le presente studio, cytochromo C esseva mesurate per medio de un multo specific methodo spectrophotometric que depende de oxydation enzymatic e reduction de iste enzyma. Rattos esseva mantenite con dietas normal o con dietas a carentia de ferro, e a intervallos appropriate illos esseva sanguinate. Un marcate reduction del contento de cytochromo C in le hepate e le renes esseva demonstrate in rattos a carentia de ferro in comparation con le rattos del gruppo de controlo. Iste reduction excedeva grandemente le leve reduction del concentration de hemoglobina. Ben que il non es permissibile derivar ab iste studios conclusiones relative al rolo de cytochromo C in anemia a carentia de ferro, illos representa un demonstration conclusive que enzymas ferrifere non es necessarimente "inviolabile," como Hahn e Whipple lo ha asserite. Iste constatation presta un certe supporto experimental al conception que le symptomatologia del carentia de ferro es possibilemente relationate a disturbationes in le metabolismo del histos plus tosto que simplemente a un reduction del hemoglobina circulante. Il es etiam possibile que le depletion de ferro sin anemia produce symptomas, sed iste importante possibilitate ha non ancora essite demonstrate.

CHANGES IN SERUM GLUTAMIC OXALACETIC TRANSAMINASE IN PATIENTS WITH DISEASES OF THE HEART, LIVER OR MUSCULOSKELETAL SYSTEMS

By Charles B. Moore, M.D.
Fellow in Internal Medicine, Alton Ochsner Medical Foundation

Robert Birchall, M.D.

Harold M. Horack, M.D.

and

Hugh M. Batson, M.D.

(From the Department of Internal Medicine, Ochsner Clinic, New Orleans, Louisiana)

The initial physiologic observations on transaminase were made by Needham[13] in 1930 and amplified by Cohen and Hekhuis[2,3] in 1940 and 1941. LaDue, Wróblewski and Karmen[6] suggested the clinical implications of serum glutamic oxalacetic transaminase (transaminase) activity in 1954. Since then, more than 50 reports dealing with the clinical significance and other aspects of variations in the serum transaminase levels have appeared in the literature. As a result it is now well established that transaminase is present in high concentrations in the heart, striated muscle, liver, kidney, and brain, and that the serum level is significantly elevated in destructive lesions of these organs and in obstructive lesions of the biliary tract. The physiochemical changes responsible for the rise in serum transaminase levels have been described in detail by others (Lemley-Stone *et al.*[8], Merrill *et al.*[11], and Nydick, Wróblewski and LaDue[14]).

The demonstration that a hitherto unexamined entity is consistently elevated or depressed in a given disease or group of diseases is always important and often exciting. It carries with it, however, the danger that in the rosy exuberance of discovery it will be promptly instituted as a routine laboratory procedure, even though the determinations, albeit consistent, actually contribute nothing to the diagnosis or treatment of a disease in an individual patient. Medical care is expensive today; yet laboratory studies are too often ordered as "batteries" when all but the first salvo serve only to increase the patient's bill, rather than to contribute information of clinical importance.

The consistent deviations of the serum transaminase levels in various pathologic states are of great interest. It seemed important to determine in exactly what situations, in our experience, the information obtained from serial transaminase determinations is of sufficient clinical importance to justify its routine use as a laboratory procedure. Accordingly, serial transaminase levels have been recorded in 173 patients with diseases of the heart, liver, musculoskeletal system or other organs. The purpose of this paper is to assess the clinical significance of serial changes in the transaminase levels, and to indicate under what circumstances they represent a helpful clinical test. Some previously unre-

ported observations of serum transaminase determinations on patients with gout and other hyperuricemic states will also be presented.

Method and Material. The method of Henley and Pollard[4] was utilized with one variation. It was observed that after protein precipitation with heat, a better yield of the supernatant fluid could be obtained by cooling the specimens in ice prior to centrifugation, and thus decrease evaporation. For economic reasons this method was initially preferred to the more commonly used procedure of Karmen, Wróblewski and LaDue[5] and it was later found to be preferable for several other reasons:

1. At two points during the procedure the test may be interrupted and finished at a later date at the convenience of the technician.
2. The results are reported in micromoles/100 ml./hr.
3. It is an inexpensive, easily performed and reliable test giving consistent results.

All determinations were obtained on patients hospitalized at the Ochsner Foundation Hospital and seen by one of us. The initial laboratory determinations and all normal determinations were obtained by one of us (C.B.M.). Each patient was subjected to a thorough physical examination and laboratory tests, including serial electrocardiograms and serial liver function tests, whenever it was considered not injurious to health. Patients with diseases that might conceivably elevate the transaminase levels were carefully excluded.

Results. 1. NORMAL SUBJECTS. Single isolated determinations were made on 100 normal subjects. The values ranged from 18 micromoles/100 ml./hr. to 119 micromoles/100 ml./hr., with a mean of 75.9 micromoles/100 ml./hr. There was no significant difference between the values for males and females in this group. The serum transaminase values in the control subjects were readily reproducible from day to day with variations of no more than 5 micromoles/100 ml./hr. in the same subject. The maximum total fluctuation in this group over a period of 9 days was 15 micromoles/100 ml./hr., and the maximum daily fluctuation was 8 micromoles/100 ml./hr. We, therefore, consider the normal transaminase range to be between 33 micromoles/100 ml./hr. and 118 micromoles/100 ml./hr., which represents the mean of 75.9 micromoles/100 ml./hr. ± twice the standard deviation of 21.1 micromoles/100 ml./hr.

2. PATIENTS WITH ABNORMAL TRANSAMINASE LEVELS. Abnormal transaminase levels were encountered in a large number of patients with diverse diseases. Results of these tests are shown in Table 1.

3. PATIENTS WITH NORMAL TRANSAMINASE LEVELS. Normal levels for serum transaminase were consistently found in a large number of patients with various diseases. The results of these tests are shown in Table 2. In addition to the conditions listed, single determinations of serum transaminase were found to be normal in patients with incomplete abortion, nephrosis, pulmonary emphysema, acromegaly, giant follicular lymphoma, and organic mental syndrome secondary to multiple cerebral infarctions.

Discussion. In our opinion, the method of Henley and Pollard is a highly accurate procedure and is preferable to the more recently introduced and less specific phenylhydrazine test. Results have been reported in micromoles/100 ml./hr. rather than in units. One "unit" represents a decrease in optical density of 0.001/ml./minute at wave length 340 millimicrons at room temperature. This may be converted to micromoles if it is assumed that the procedure is performed at a constant reasonable temperature. In contradistinction, we permitted the enzymatic reaction in the spectrophotometer to continue to completion and then calculated the weight in micromoles of oxalacetic acid produced. This permits direct comparison with other methods,

and with the same method performed in different laboratories.

NORMAL SUBJECTS. The normal range was established as 33 to 118 micromoles/100 ml./hr. at 37° C. Such a wide range introduces a possible error in interpretation that can be avoided only if enough serial determinations are obtained to establish the true normal for an individual. In fact, although it has not been clearly established, it seems likely that a rise from a base line of 40 micromoles/100 ml./hr. to 100 micromoles/100 ml./hr. is

TABLE 1.—ABNORMAL SERUM TRANSAMINASE LEVELS IN VARIOUS DISEASES

			Serum Transaminase Level (Normal 75.9 micromoles/100 ml./hr.)			
Diagnosis	*No. of Patients*	*No. of Tests*	*Minimum*	*Mean*	*Maximum*	*Duration of Change*
Myocardial infarction	35	490	40	—	1280	3 to 10 days
Acute coronary insufficiency	15	193	38	—	220	1 to 3 days
Cardiac surgery	6	48	47	—	390	1 to 2 days
Cardiac catheterization	5	20	37	—	137	6 hours
Active Laennec's cirrhosis	7	35	173	472	1280	Constant
Infectious hepatitis	5	50	305	—	2429	4 weeks or more
Metastatic carcinoma to liver	5	10	135	183	265	Constant
Acute pancreatitis	3	16	133	260	410	3 weeks
Common bile duct obstruction	3	20	117	243	450	Until obstruction relieved
Hemochromatosis	2	6	320	441	568	Constant
Portacaval shunts	2	8	77	—	781	Fell post-operatively
Cholangiolytic hepatitis	1	4	152	164	180	Constant
Wilson's disease	1	4	248	270	310	Constant
Biliary cirrhosis	1	3	237	251	274	Constant
Gout	20	41	74	—	225	Elevated with acute attack
Muscle biopsy	5	10	72	—	300	Elevated post-operatively
Acute leukemia	1	2	144	—	148	Constant

TABLE 2.—NORMAL SERUM TRANSAMINASE LEVELS IN VARIOUS DISEASES

			Serum Transaminase Level (Normal 75.9 micromoles/100 ml./hr.)		
Diagnosis	*No. of Patients*	*No. of Tests*	*Minimum*	*Mean*	*Maximum*
Angina pectoris	10	20	48	81	104
Chest or arm pain of extracardiac origin	5	12	57	78	114
Pericarditis	1	10	104	107	111
Inactive Laennec's cirrhosis	4	22	49	84	113
Carcinoma without metastases to liver	4	8	61	90	107
Rheumatoid arthritis	11	19	43	79	99
Scleroderma	1	4	77	81	88
Lupus erythematosus	4	9	76	90	110
Venous cutdowns	5	10	64	80	87
Polycythemia vera	8	16	39	74	114

just as significant as a rise from 100/micromoles/100 ml./hr. to 160 micromoles/100 ml./hr., although only the last value is abnormal by the present standards.

HEART DISEASE. The characteristic transaminase curve in acute myocardial infarction is well delineated (Fig. 1) (Chinsky, Shmagranoff and Sherry[1] LaDue and Wróblewski[7], and Merrill *et al.*[10]). To establish the diagnosis and follow the clinical course of a classical attack of myocardial infaction, serial transaminase determinations probably contribute nothing, and for that reason, should not be ordered routinely. Their chief clinical value would appear to be in:

1. The early recognition of necrosis of the myocardium in patients seen for the first time with substernal pain whose electrocardiogram:

a) shows only left bundle branch block,

b) is not diagnostic, and either intramural or subendocardial infarction is suspected, or

c) fails to show a pattern characteristic of myocardial infarction, in which case it will serve as a clear indication of the need for additional leads with the use of an exploring electrode;

d) does not show an infarct because it is located in a region inaccessible to the standard commonly used exploring electrode;

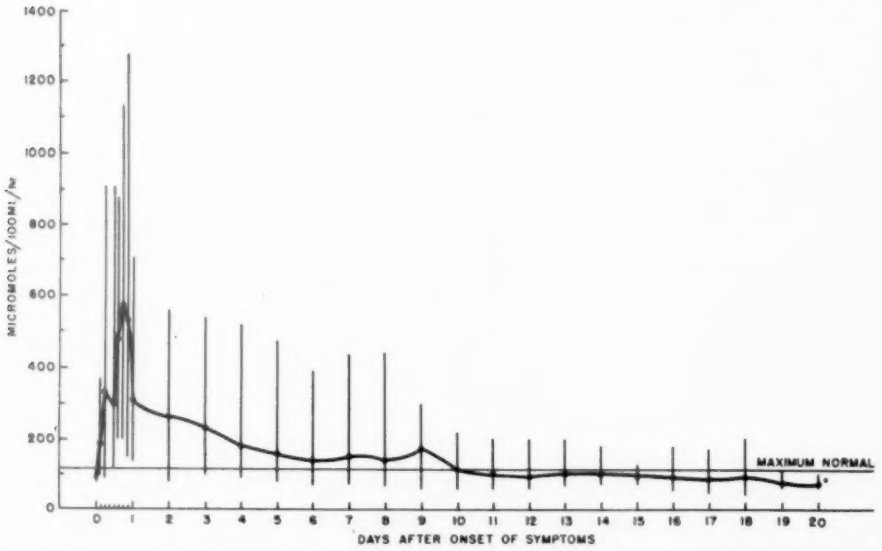

Fig. 1.—Transaminase levels in acute myocardial infarction. The transaminase level is elevated within 6 hours after onset of symptoms, reaches its peak in 18 hours and returns to normal in 10 days.

2. Early recognition of myocardial necrosis in localities where competent electrocardiographic interpretation is not immediately available, or in which the exact interpretation of an unusual electrocardiogram is in doubt;

3. Occasional detection of extension of an infarction during observation and treatment of a patient (Fig. 2).

The transient, slight but definite rise in the serum transaminase level in patients with acute coronary insufficiency

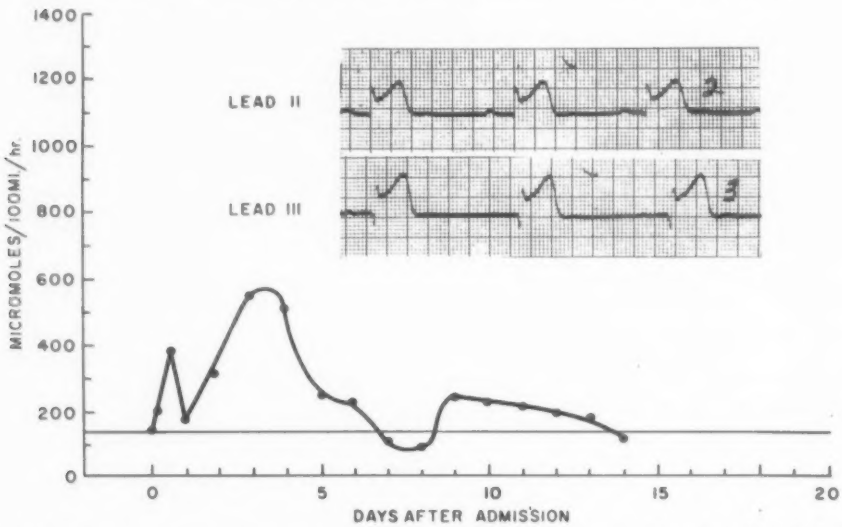

Fig. 2.—The transaminase curve on a patient who was admitted with the clinical and electrocardiographic diagnosis of acute posterior myocardial infarction. Thirty-six hours after admission the electrocardiogram revealed extension of the infarction anteriorly through the septum. On the tenth hospital day, various arrhythmias developed and, after normal sinus rhythm had been re-established, the electrocardiogram revealed changes consistent with further extension of the infarction of the posterior wall. The serum transaminase levels rose during the time of both extensions of the infarction.

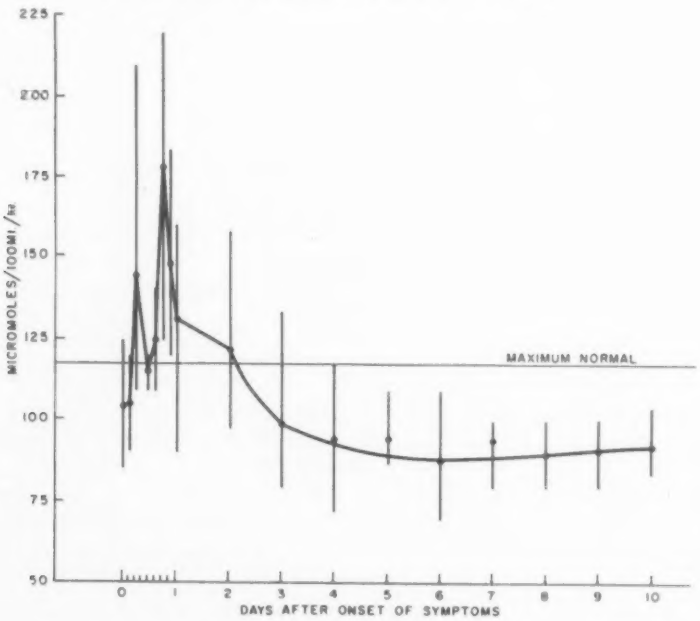

Fig. 3.—Transaminase curve obtained in patients with acute coronary insufficiency. The onset of the rise, as well as the peak reached, corresponds in time with that observed in classical myocardial infarction, but was not of the same magnitude. The maximum rise was 220 micromoles/100 ml./hr. in 18 hours. The average peak was 178 micromoles/100 ml. hr., a value considerably higher than the normal limits.

(Fig. 3) lends credence to the idea that in such patients there is actually some infarction of the myocardium (Master *et al.*[9]). This is in contradistinction to patients with angina pectoris whose transaminase levels do not deviate from the base line.

Of interest is the slight increase in serum transaminase levels of patients who had mitral commissurotomy, in contrast to the precipitous elevation in those who had undergone resection of a ventricular aneurysm or closure of an interatrial septal defect by the

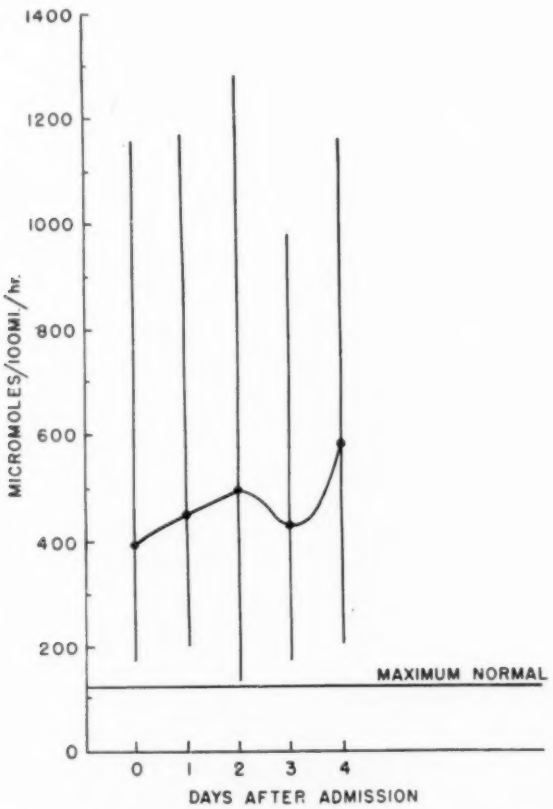

Fig. 4.—Range of transaminase values in patients with active Laennec's cirrhosis.

The serum transaminase level is often of real value in the differentiation of pain of cardiac injury from the cardiac-like pains of extracardiac origin, compression of the cervical nerve roots, esophageal spasm, cervical osteoarthritis, reaction to cocaine, cardiac neurosis and many others (Riseman[15]).

Sondergaard circumclusion technique. With the latter technique a heavy ligature is passed completely around the entire interatrial septum. Constriction of this ligature, of sufficient extent to close a septal defect, probably interferes with the blood supply to the septal musculature.

Also of interest is the fact that 12

hours after cardiac catheterization the transaminase level was elevated about 40 micromoles/100 ml./hr. above the previously normal base line. This appears to be due to myocardial injury incident to passage of the catheter through the heart, since it is not elevated after a venous cutdown procedure, or after insertion of a cardiac catheter into the superior vena cava.

HEPATIC DISEASE. It has been well established that destruction of hepatic cells by any cause results in a rise in the serum transaminase level (Molander, Wróblewski and LaDue[12]). Thus, the serum transaminase level is elevated in active cirrhosis (Fig. 4), hemochromatosis, Wilson's disease, biliary cirrhosis, and various types of hepatitis, and, therefore, may serve as evidence of activity of disease, particularly in the differentiation of active from inactive cirrhosis.

The test may also be of great value in the distinction of obstructive jaundice from that due to hepatocellular disease. In our patients with obstructive jaundice the transaminase level never exceeded 320 micromoles/100 ml./hr., whereas in hepatocellular disease (Fig. 5), during the active stages, the average transaminase level was in excess of 1000 micromoles/100 ml./hr. In two instances, operation was cancelled on patients suspected of having obstructive jaundice because of transaminase levels in excess of 2000 micromoles/100 ml./hr. Subsequently, both were proved to have had active infectious hepatitis, either by liver biopsy or at necropsy.

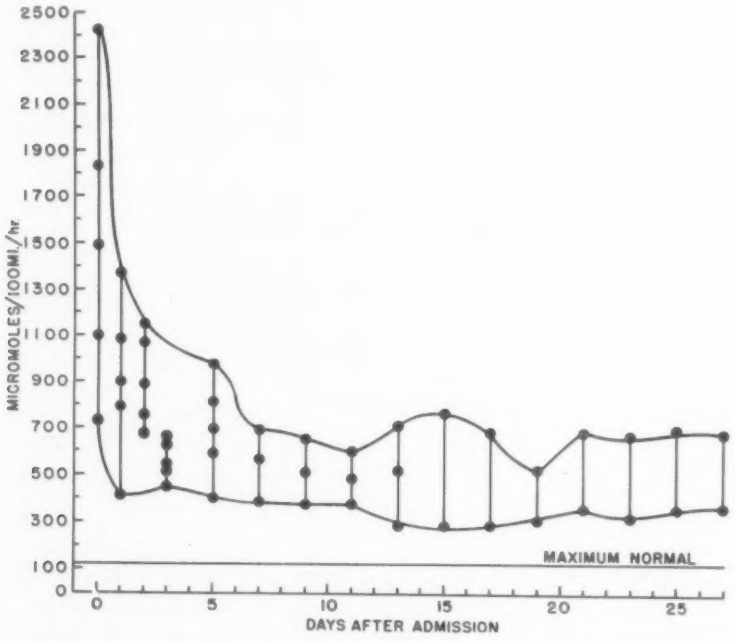

Fig. 5.—Range of transaminase values in patients with infectious hepatitis.

That the transaminase levels were elevated in all patients suspected of having metastasis to the liver seems significant. At present it is not possible to state categorically that an elevated

serum transaminase level in a patient with known carcinoma implies metastasis to the liver. However, if the serum transaminase level is normal, metastatic disease of the liver is certainly less likely. This may serve as one factor in determining the extent of a contemplated surgical procedure.

Because the serum transaminase level is elevated in both pancreatitis and cholelithiasis with obstruction of the common bile duct, it is of little value in differential diagnosis. However, it is valuable in determining whether or not obstruction of the common bile duct from any cause has been completely relieved, for, after relief of obstruction, the serum transaminase level should promptly return to normal.

We have no explanation for the return of the transaminase level to normal within 7 days after portacaval shunt in the 2 patients with hepatic cirrhosis and portal hypertension. There is no reason to believe that anything other than the portal hypertension had been relieved. Possibly, the mechanism of the fall in the transaminase levels to normal is the same as that after treatment of a patient with congestive heart failure and relief of hepatic congestion.

MUSCULOSKELETAL DISEASES. Transaminase levels were consistently normal in rheumatoid arthritis, scleroderma and lupus erythematosus. Extreme elevation of the transaminase level was noted in 2 of our patients with dermatomyositis, confirmed by biopsy and necropsy. This observation has also been made by Siekert and Fleisher[16]. After treatment with steroids the disease became quiescent in one of the patients, and the transaminase level fell to normal.

In sharp contradistinction to patients with rheumatoid arthritis, all patients with gout had elevated transaminase levels at some time during their hospitalization. In 14 of the 20 patients the transaminase level was between 175 and 225 micromoles/100 ml./hr., at a time when the serum uric acid was elevated. However, there was no apparent direct relationship between the serum transaminase, the concentration of uric acid in the serum, and the activity of the gout. The relationship between the serum transaminase and the serum uric acid level in patients with gout, leukemia, polycythemia, hyperuricemia, or myocardial infarction (in whom we have found consistently elevated uric acid levels) is at present under investigation.

It should be noted that the significant rise in serum transaminase after simple muscle biopsy should not be mistakenly interpreted as evidence of pathologic destruction of muscle.

MISCELLANEOUS. The serum transaminase levels were normal in the remainder of the patients studied.

Summary and Conclusions. 1) The results of 1284 serum transaminase determinations performed serially upon 173 patients and 100 normal subjects have been analyzed.

2) The method of Henley and Pollard, with one slight improvement, was found to be inexpensive, accurate and simple.

3) The results of serial transaminase determinations have been of significant clinical value in diseases of the

A. HEART.

1. Early recognition of necrosis of myocardium in patients seen for the first time with thoracic pain whose electrocardiogram a) shows only left bundle branch block, b) is not diagnostic and either an intramural or a subendocardial infarction is suspected, c) fails to show a pattern characteristic of myocardial infarction, which serves as a clear indication of the need for additional leads with the use of an

exploring electrode, d) fails to show an infarct because it is located in a region inaccessible to the standard commonly used exploring electrodes.

2. Early recognition of myocardial necrosis in localities where competent electrocardiographic interpretation is not immediately available, or in which the exact interpretation of an atypical electrocardiogram is in doubt.

3. Occasional detection of extension of an infarction during observation and treatment of a patient.

4. Differentiation of acute myocardial infarction, acute coronary insufficiency and angina pectoris.

5. Confirmation of the clinical impression that thoracic pain in some patients is of extracardiac origin.

B. LIVER.

1. Differentiation of obstructive from hepatocellular jaundice.

2. Presence of activity in parenchymal disease of the liver.

3. Demonstration of relief of obstruction of the common bile duct.

4. Strong evidence that there is no metastasis to the liver in a patient with known carcinoma elsewhere.

C. MUSCULOSKELETAL SYSTEM.

1. Differentiation of dermatomyositis from other collagen diseases.

4) The hitherto unreported observation has been made that the serum transaminase level is elevated in patients with gout as well as in patients with other causes of hyperuricemia (myocardial infarction, leukemia and polycythemia vera). There is apparently no direct relationship between the serum transaminase level and the blood uric acid levels, but further study of this aspect of the problem is underway at present.

REFERENCES

1. **Chinsky, M., Shmagranoff, G. L.,** and **Sherry, S.:** J. Lab. & Clin. Med., **47,** 108, 1956.
2. **Cohen, P. P.,** and **Hekhuis, G. L.:** J. Biol. Chem., **136,** 565, 1940.
3. Iidem: Ibid., **140,** 711, 1941.
4. **Henley, K. S.,** and **Pollard, H. M.:** J. Lab. & Clin. Med., **46,** 785, 1955.
5. **Karmen, A., Wróblewski, F.,** and **LaDue, J. S.:** J. Clin. Invest., **34,** 126, 1955.
6. **LaDue, J. S., Wróblewski, F.,** and **Karmen, A.:** Science, **120,** 497, 1954.
7. **LaDue, J. S.,** and **Wróblewski, F.:** Circulation, **11,** 871, 1955.
8. **Lemley-Stone, J., Merrill, J. M., Grace, J. T.,** and **Meneely, G. R.:** Am. J. Physiol., **183,** 555, 1955.
9. **Master, A. M., Jaffe, H. L., Dack, S.,** and **Grishman, A.:** Am. Heart J., **27,** 803, 1944.
10. **Merrill, J. M., Lemley-Stone, J., Grace, J. T., Jr.,** and **Meneely, G. R.:** J. Am. Med. Assn., **160,** 1454, 1956.
11. **Merrill, J., Stone, J. L., Grace, J. T.,** and **Meneely, G. R.:** Clin. Rec. Proc., **3,** 21, 1955.
12. **Molander, D., Wróblewski, F.,** and **LaDue, J. S.:** J. Lab. & Clin. Med., **46,** 831, 1955.
13. **Needham, D. M.:** Biochem. J., **24,** 208, 1930.
14. **Nydick, I., Wróblewski, F.,** and **LaDue, J. S.:** Circulation, **12,** 161, 1955.
15. **Riseman, J. E. F.:** Ibid., **14,** 422, 1956.
16. **Siekert, R. G.,** and **Fleisher, G. A.:** Proc. Staff Meet., Mayo Clin., **31,** 459, 1956.

SUMMARIO IN INTERLINGUA

Alterationes in Nivellos Seral de Transaminase Glutamic-Oxalacetic in Patientes con Morbos de Corde, Hepate, o Systemas Musculoskeletic

1) Es analysate le resultatos de 1284 determinationes de transaminase seral, effectuate in series in 173 patientes e 100 subjectos normal.

2) Esseva trovate que le methodo de Henley e Pollard, con un sol e non-extense melioration, es incostose, accurate, e simple.

3) Le resultatos del determinationes serial de transaminase esseva de significative valor clinic in varie morbos.

A. CORDE.

1. Plus prompte recognition de necrosis del myocardio in patientes vidite le prime vice con dolores thoracic e in qui le electrocardiogramma (a) monstra solmente bloco del branca sinistre, (b) non es diagnostic, durante que il existe le suspicion de un infarcimento intramural o subendocardial, (c) non exhibi un configuration characteristic de infarcimento myocardial, un facto que indica clarmente le necessitate de derivationes additional con le utilisation de un electrodo expolorante, o (d) monstra nulle infarcimento proque le sito del infarcimento es inaccessibile al standard electrodos explorante que es in uso commun.

2. Prompte recognition de necrosis myocardial quando le experte interpretation del electrocardiogramma non es immediatemente disponibile o quando le interpretation de un electrocardiogramma atypic es dubitose.

3. In casos sporadic, le detection del extension de un infarcimento durante le observation e tractamento del patiente.

4. Differentiation inter acute infarcimento myocardial, acute insufficientia coronari, e angina de pectore.

5. Confirmation del impression clinic que le dolores thoracic de certe patientes es de origine extracardiac.

B. HEPATE.

1. Differentiation inter jalnessa obstructive e jalnessa hepatocellular.

2. Presentia de activitate in morbo parenchymic del hepate.

3. Demonstration de alleviamento del obstruction in le ducto biliari commun.

4. Forte supporto del conclusion que metastases hepatic es absente in patientes con carcinoma demonstrate in altere partes del corpore.

C. SYSTEMA MUSCULOSKELETIC.

1. Differentiation de dermatomyositis ab altere morbos de collageno.

4) Esseva facite le usque nunc non reportate observation que le nivello seral de transaminase es elevate in patientes con gutta si ben como in patientes con altere causas de hyperuricemia (infarcimento myocardial, leucemia, e polycythemia ver). Apparentemente il non existe un relation directe inter le nivello seral de transaminase e le nivellos de acido uric in le sanguine, sed studios additional de iste aspecto del problema es currentemente in progresso.

CHANGES IN SERUM PSEUDO-CHOLINESTERASE LEVELS IN PATIENTS WITH DISEASES OF THE HEART, LIVER OR MUSCULOSKELETAL SYSTEMS

By Charles B. Moore, M.D.
FELLOW IN INTERNAL MEDICINE, ALTON OCHSNER MEDICAL FOUNDATION

Robert Birchall, M.D.

Harold M. Horack, M.D.

AND

Hugh M. Batson, M.D.

(From the Department of Internal Medicine, Ochsner Clinic, New Orleans, Louisiana)

Serum pseudo-cholinesterase (cholinesterase) tests have been performed for a number of years upon patients with a variety of diseases (Antopol, Tuchman and Schifrin[1,2], Faber[4]). The original procedure was laborious and expensive, and consequently rarely performed. In 1948, Michel[6] introduced a simple accurate test for determining the serum and erythrocyte cholinesterase level. Since then, the concentration of this enzyme in the serum has been studied chiefly in diseases of the liver, as it has been shown that the serum cholinesterase measured by this technique is formed in the liver (Sawyer and Everett[10]).

In 1954, Oka[9] reported significant changes in the serum cholinesterase levels, performed serially, after acute myocardial infarction in 7 patients. In our previous investigation of serial serum glutamic oxalacetic transaminase levels in a variety of diseases[8], it was believed that the same blood samples could be used to determine cholinesterase levels and thus permit a study of the relative merits of the two procedures. The purpose of this paper, therefore, is to illustrate the clinical value of serial changes in the cholinesterase levels, to compare the results with concomitant changes in serum transaminase, and to indicate under what circumstances changes in cholinesterase levels represent a valid and helpful clinical test.

Method and Material. The method of Michel[6] was employed, serum having been utilized instead of plasma. The pH of the solution in the potentiometer was determined at one-minute intervals for 90 minutes. This revealed that the reaction proceeded in a straight line, and that any two points on the line sufficiently far apart for an accurate reading, could be used to delineate the slope of the line and, therefore, the concentration of cholinesterase in the serum. In order to conserve time we arbitrarily selected initial and 30-minute readings as the base line for our calculations. The results of this procedure were easily reproducible on the same serum, with a day to day variation of less than 0.05 delta pH/ hr.

All determinations were made on patients seen by one of us at the Ochsner Foundation Hospital. The initial laboratory determinations and all normal determinations were performed by one of us (C.B.M.). An attempt was made to obtain the specimens while the subject was fasting, and this was done in the normal group, but in the others, because symptoms developed in many instances, this was not always possible. Whenever it was not injurious to health, the patient had thorough physical and laboratory evaluation, including serial electrocardiograms and serial liver function tests. Patients with associated diseases that might conceivably alter the cholinesterase levels were carefully excluded.

Results. 1. NORMAL PERSONS. Single, isolated determinations were obtained on 100 normal persons. The values ranged from 0.48 delta pH/hr. to 1.24 delta pH/hr., with a mean of 0.785 delta pH/hr., corresponding with the normal values obtained by others (Molander, Friedman and LaDue[7], and Vorhaus, Scudamore and Kark[11]). There was no significant difference between the values in males and females. Serial determinations were secured on 5 normal subjects; the maximum daily fluctuation was 0.10 delta pH/hr., and the maximum total fluctuation during a period of 9 days was 0.21 delta pH/hr. We, therefore, consider the normal serum cholinesterase level to be between 0.50 delta pH/hr. and 1.07 delta pH/hr., which represents the mean of 0.785 delta pH/hr. ± twice the standard deviation of 0.142 delta pH/hr.

2. PATIENTS WITH ABNORMAL CHOLINESTERASE LEVELS. Abnormal, or changing, levels of serum cholinesterase were consistently found in patients with certain diseases of the heart, liver or musculoskeletal system. The results of serial serum cholinesterase levels in these patients are shown in Table 1.

3. PATIENTS WITH NORMAL CHOLINESTERASE LEVELS. Normal values for serum cholinesterase were consistently found in a large number of patients. The results of these tests are shown in Table 2. In addition to the conditions listed, normal cholinesterase levels were noted in patients with incomplete abortion, nephrosis, acromegaly, giant follicular lymphoma, organic brain syndrome secondary to multiple cerebral infarction, and duodenal ulcer.

TABLE 1.—ABNORMAL CHOLINESTERASE LEVELS IN VARIOUS DISEASES

Diagnosis	*No. of Patients*	*No. of Tests*	*Cholinesterase Level (Normal, 0.785 Δ pH/hr.)*			*Duration of Change*
			Minimum	*Maximum*	*Mean*	
Myocardial infarction with survival	26	364	0.18	1.34	—	3 to 5 days
Fatal myocardial infarction	4	56	0.12	0.75	—	Until death
Cardiac surgery	6	48	0.30	1.00	—	3 to 5 days
Active Laennec's cirrhosis	7	35	0.08	0.83	0.241	Constant
Infectious hepatitis	5	50	0.08	0.64	—	3 to 4 weeks
Metastatic carcinoma to the liver	5	10	0.14	0.35	0.239	Constant
Hemochromatosis	2	6	0.95	1.17	1.08	Constant
Cholangiolytic hepatitis	1	4	0.35	0.47	0.41	Constant
Wilson's disease	1	4	0.35	0.41	0.39	Constant
Dermatomyositis	2	7	0.19	0.25	0.21	Constant
Acute lymphatic leukemia	1	2	0.21	0.25	0.23	Constant
Portacaval shunts	2	8	0.40	0.85	—	Postoperatively

Discussion. Michel's method for determining cholinesterase concentration proved to be simple, accurate and inexpensive. The enzyme measured in the serum by this technique, "pseudo" cholinesterase, is present in liver, pancreas and serum (Best and Taylor[3]). Its function has not yet been determined. It differs in many respects from "true" cholinesterase, which is found in the brain, red blood cells, muscle

and nerves. True cholinesterase is found in high concentrations at the motor end plate and thus, whenever acetylcholine is formed at a motor end plate to activate nerve tissue, "true" cholinesterase is also present to terminate its action (Best and Taylor[3]).

Pseudo-cholinesterase is a large molecular mucoprotein that has been associated with the albumin fraction of the serum proteins (Sawyer and Everett[10]). It is relatively insensitive to eserine, but rapidly inactivated by di-isopropylfluorophosphate. Pseudo-cholinesterase functions to de-acetylate acetylcholine to acetic acid and choline chloride at high concentrations of acetylcholine, but at low (physiologic) concentrations, the true cholinesterase is much more active (Best and Taylor[3]). Pseudo-cholinesterase is apparently formed in the liver and has a life span of 28 days (Sawyer and Everett[10]). In general, hepatic diseases that lower the serum albumin result in a lowered serum pseudo-cholinesterase level also. In contrast, in nephrosis in which the albumin is low despite a normal or exaggerated rate of synthesis, the serum pseudo-cholinesterase, being a larger, relatively unfilterable molecule, remains at normal levels.

NORMAL SUBJECTS. The normal range of serum cholinesterase values has been established as being from 0.50 delta pH/hr. to 1.07 delta pH/hr. We believe, however, that for practical purposes any value above 0.50 delta pH/hr. can be considered as normal.

TABLE 2.—NORMAL CHOLINESTERASE LEVELS IN VARIOUS DISEASES

Diagnosis	*No. of Patients*	*No. of Tests*	*Cholinesterase Level (Normal, 0.785 Δ pH/hr.)*		
			Minimum	*Maximum*	*Mean*
Acute coronary insufficiency	12	154	0.62	1.10	0.84
Angina pectoris	10	20	0.75	0.98	0.85
Extracardiac chest or arm pain	5	12	0.74	1.03	0.88
Cardiac catheterization	5	20	0.71	1.11	0.88
Pericarditis	1	10	0.66	0.74	0.70
Inactive Laennec's cirrhosis	4	22	0.45	0.60	0.55
Carcinoma without metastasis to the liver	4	8	0.44	0.71	0.59
Acute pancreatitis	3	16	0.51	0.94	0.74
Acute cholecystitis with cholelithiasis	3	20	0.44	1.01	0.851
Rheumatoid arthritis	11	19	0.47	1.04	0.794
Gout	10	20	0.54	1.07	0.84
Scleroderma	1	4	0.66	0.71	0.68
Lupus erythematosus	4	9	0.54	0.97	0.74
Venous cutdowns	5	10	0.61	0.99	0.84
Muscle biopsy	5	10	0.51	0.88	0.64
Polycythemia vera	2	4	0.66	0.89	0.74
Hyperthyroidism	2	6	0.77	0.96	0.88

HEART DISEASE. The characteristic cholinesterase curve in acute myocardial infarction has not been well established, although in 1954 Oka[9] reported that the serum cholinesterase concentration fell following acute myocardial infarction and rose as recovery proceeded. He suggested that serial determinations of serum cholinesterase might be of prognostic import. Our studies show (Fig. 1) that the serum cholinesterase level falls after acute myocardial infarction, but that time relation and degree of fall vary so greatly from patient to patient that

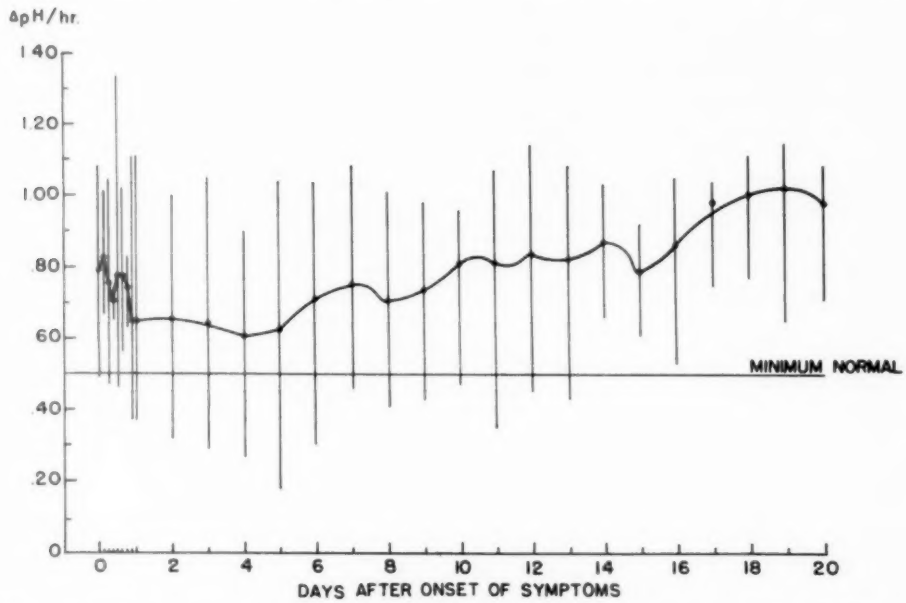

Fig. 1.—Acute myocardial infarction with survival. There was an immediate fall in the mean cholinesterase value, the lowest level being reached on the fourth day after onset of symptoms with return to normal by the twelfth day.

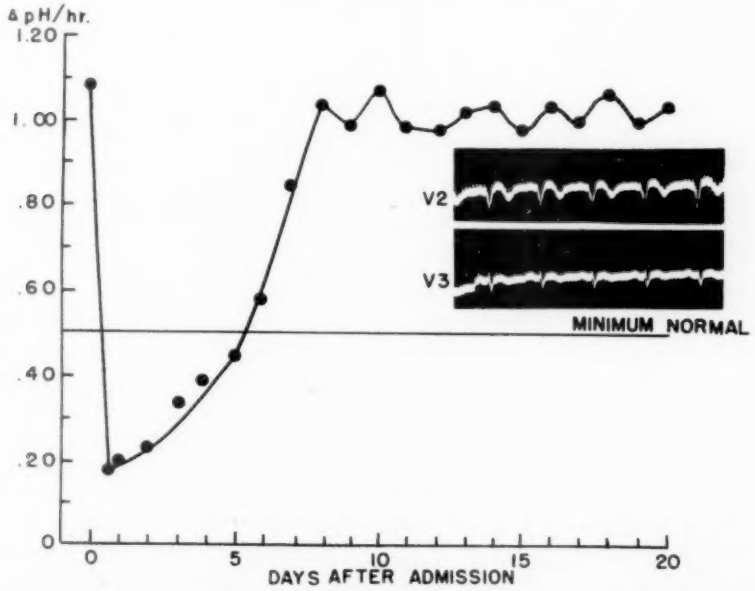

Fig. 2.—Cholinesterase curve obtained in a patient with an acute anteroseptal myocardial infarction considered to be extensive by clinical evaluation. The serum cholinesterase concentration fell 0.90 delta/pH/hr. initially and then returned to a normal limit on the eighth day after onset of symptoms.

interpretation may be difficult if not impossible (Fig. 2).

The most significant observation appeared to be that the serum cholinesterase level progressively fell in patients who eventually died, and reached the lowest level just before death (Fig. 3). This suggests that a progressively falling serum cholinesterase level after acute myocardial infarction is of grave prognostic import (Fig. 4).

level does not occur until the fourth day after onset of symptoms.

A simple explanation for the fall in serum cholinesterase levels after myocardial infarction is lacking. The usual explanations for a fall in the serum concentration of any substance include increased destruction, decreased synthesis and increased excretion of the substance, and prevention of its appearance in the serum from its source.

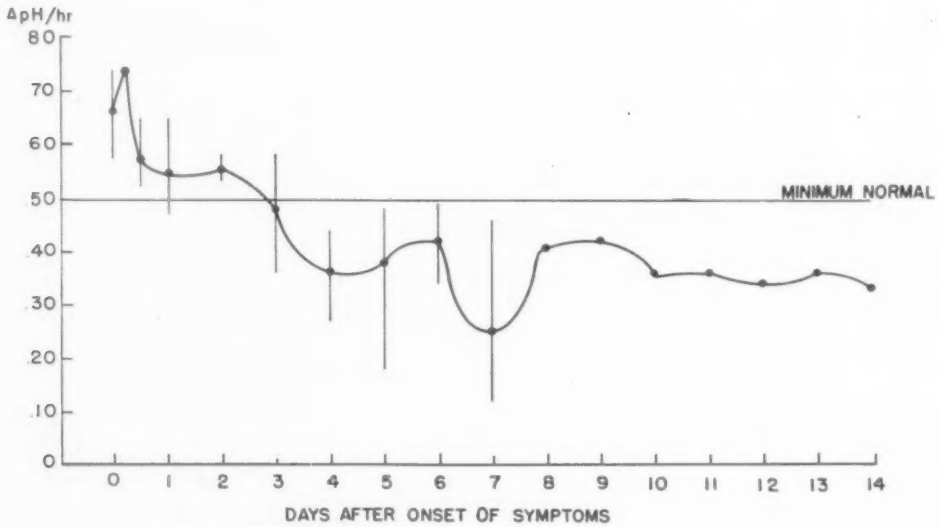

Fig. 3.—Fatal acute myocardial infarction. The mean cholinesterase value dropped gradually, the lowest level having been reached at death.

As an index of myocardial damage, serum cholinesterase determinations alone are less sensitive than serum glutamic oxalacetic transaminase levels. Thus, in acute myocardial insufficiency and in cardiac catheterizations, serum transaminase determinations rise significantly, but serum cholinesterase levels do not change. Furthermore, serum transaminase levels are elevated 6 hours after onset of symptoms in acute myocardial infarction and reach their peak in 18 hours, whereas the maximum drop in serum cholinesterase

It is difficult to determine which mechanism causes the fall in serum cholinesterase levels in patients with myocardial infarctions, but some can be easily excluded. With the demonstration that the serum cholinesterase has a life span of 28 days, both decreased synthesis of the enzyme, and failure of the enzyme to appear in the blood from its source of formation, are unlikely. The other possibilities cannot yet be assessed. The demonstration that administration of ACTH to normal subjects (Kaufman[5]) results in a fall

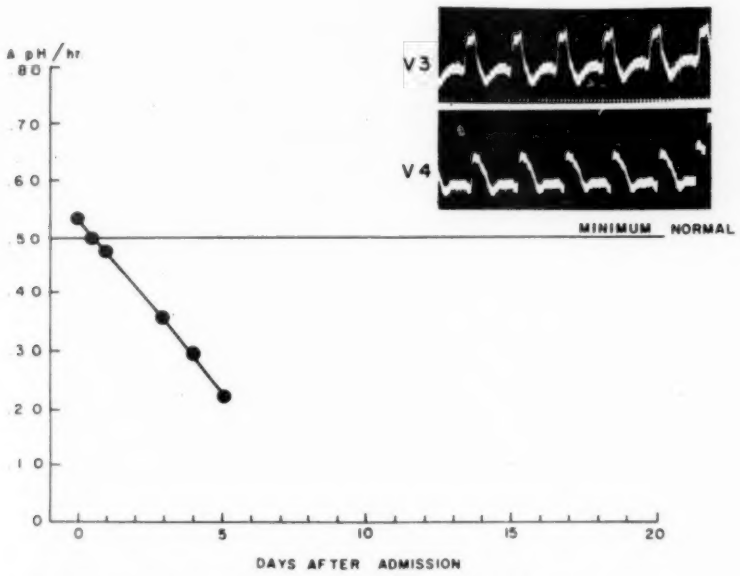

Fig. 4.—Cholinesterase levels obtained in a patient with fatal acute anterior myocardial infarction.

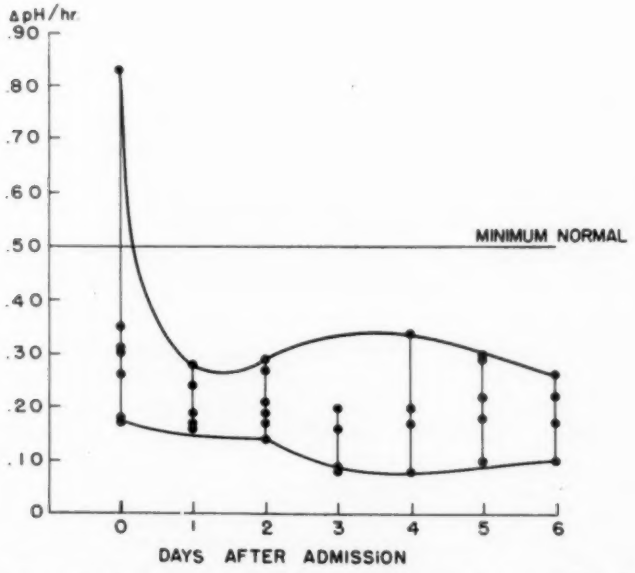

Fig. 5.—Range of values for serum cholinesterase observed in patients with active Laennec's cirrhosis.

in serum cholinesterase, and the fact that after periods of physical stress the serum cholinesterase level is low, suggests that the mechanism of the fall in myocardial infarction might be simply the result of stress. This cannot be proved at present.

As would be expected, serum cholinesterase levels remained normal in patients with angina pectoris, benign pericarditis, or pain in the chest or arm of extracardiac origin.

Immediately after repair of interatrial septal defects and after mitral commissurotomy, there was a definite rise in serum cholinesterase levels, which was maintained for 12 hours and then gradually fell; a minimum level was reached in 3 to 4 days postoperatively, after which there was a gradual return to the preoperative levels. The rise was not observed after resection of a ventricular aneurysm. We have no explanation for this.

HEPATIC DISEASE. In patients with active cirrhosis (Fig. 5), Wilson's disease, hepatitis, and carcinoma with metastasis to the liver, the serum cholinesterase concentration fell below the limits of normal. It remained within normal limits in those with inactive cirrhosis, carcinoma without demonstrable hepatic metastasis, and primary biliary cirrhosis. It was actually elevated in patients with proved hemochromatosis. The most impressive deviation was the progressive decline in serum cholinesterase levels in fatal cases of infectious hepatitis.

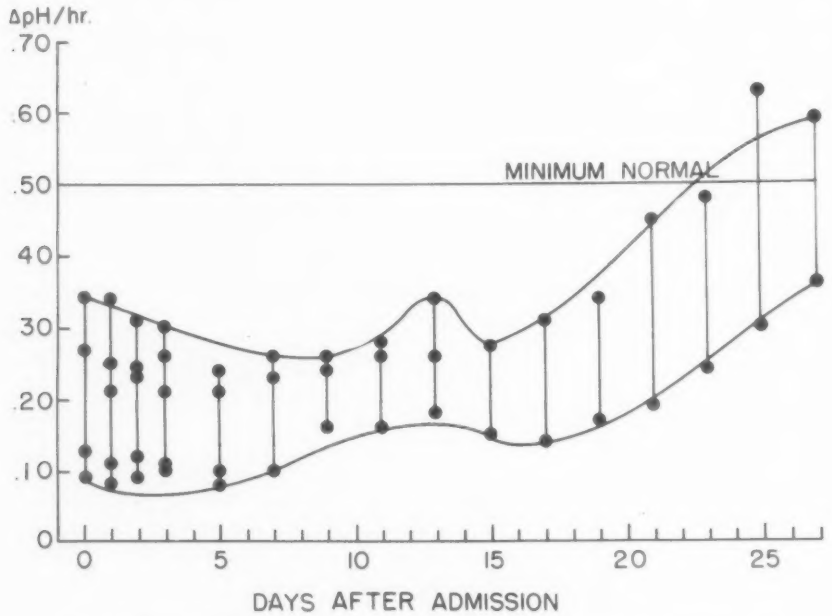

Fig. 6.—Range of values for serum cholinesterase observed in patients with infectious hepatitis.

Perhaps the chief clinical value of serum cholinesterase determinations lies in the differentiation of active hepatocellular disease, in which this value is consistently low (Fig. 6).

from obstructive jaundice, in which it is consistently normal.

In 2 patients on whom portacaval shunt was performed the serum cholinesterase levels, which were depressed preoperatively, rose to normal limits within the first week postoperatively. Presumably this is a manifestation of a decrease in portal pressure.

MUSCULOSKELETAL SYSTEM. The serum cholinesterase levels were normal in patients with rheumatoid arthritis, scleroderma, gout and lupus erythematosus. The only abnormality was a consistently low level in patients with dermatomyositis.

MISCELLANEOUS. Serum cholinesterase levels were normal in patients with polycythemia vera, hyperthyroidism, incomplete abortion, nephrosis, acromegaly, giant follicular lymphoma, organic brain syndrome secondary to multiple cerebral infarctions and duodenal ulcer. The levels were also normal after venous cutdowns and muscle biopsies. Low values were found in one patient with acute lymphatic leukemia and in one with carcinoma of the lung and metastasis to the thoracic wall. In each instance, cachexia and impending death would appear to explain adequately the low values.

Summary and Conclusions. 1. The results of 1089 serum cholinesterase determinations performed serially on 160 patients and 100 normal subjects have been analyzed.

2. The method of Michel was found to be inexpensive, accurate and simple.

3. The results of serial determinations have been of significant clinical value in diseases of the:

A. HEART. 1. The only value appears to be prognostic, since a persistently falling serum cholinesterase level occurred only in patients who did not survive.

2. It has no value in the routine management of a patient with a classical myocardial infarction who is responding favorably to therapy.

B. LIVER. 1. The serum cholinesterase levels are normal in obstructive diseases of the biliary tract and depressed in active hepatocellular disease, and appear to be more sensitive than the serum transaminase level in differentiating these.

2. The serum cholinesterase levels are helpful in indicating the presence of activity in parenchymal disease of the liver.

3. Normal serum cholinesterase levels offer suggestive evidence of the absence of metastasis to the liver in a patient with known carcinoma elsewhere.

C. MUSCULOSKELETAL SYSTEM. 1. The serum cholinesterase test is of value in differentiating dermatomyositis from other collagen diseases.

4. In general, in those diseases in which there is an increase in serum transaminase levels, there is a fall in serum cholinesterase levels. However, with respect to degree and time of change, the disparity in the curves of the two enzymes indicates that there is no true parallel in their behavior and that they bear no relation to each other. Interpretation of any single serum cholinesterase determination is impossible because of the large normal range, and interpretation of even serial determinations is difficult because of its lack of specificity. Yet, as already stipulated, in certain situations serial cholinesterase determinations may be of real clinical value.

REFERENCES

1. **Antopol, W., Tuchman, L.,** and **Schifrin, A.:** Proc. Soc. Exper. Biol. & Med., **36,** 46, 1937.
2. Iidem: Ibid., **38,** 363, 1938.

3. **Best, C. H.,** and **Taylor, N. B.:** Physiological Basis of Medical Practice, 5th ed. Baltimore: Williams & Wilkins Co., 1950.
4. **Faber, M.:** Acta med. Scandinav., **114,** 59, 1943.
5. **Kaufman, K.:** Ann. Int. Med., **41,** 533, 1954.
6. **Michel, H. O.:** J. Lab. & Clin. Med., **34,** 1564, 1949.
7. **Molander, D. W., Friedman, M. M.,** and **LaDue, J. S.:** Ann. Int. Med., **41,** 1139, 1954.
8. **Moore, C. B., Birchall, R., Horack, H. M.,** and **Batson, H. M.:** Am. J. Med. Sci., **234,** 528, 1957.
9. **Oka, M.:** Acta med. Scandinav., **150,** 313, 1954.
10. **Sawyer, C. H.,** and **Everett, J. W.:** Am. J. Physiol., **148,** 675, 1947.
11. **Vorhaus, L. J. H., Scudamore, H. H.,** and **Kark, R. M.:** Gastroenterology, **15,** 304, 1950.

SUMMARIO IN INTERLINGUA

Alterationes in Nivellos Seral de Pseudo-Cholinesterase in Patientes con Morbos de Corde, Hepate, o Systemas Musculoskeletic

1) Es analysate le resultatos de 1089 determinatones de cholinesterase del sero, effectuate in series in 160 patientes e 100 subjectos normal.

2) Esseva trovate que le methodo de Michel es incostose, accurate, e simple.

3) Le resultatos del determinationes serial esseva de significative valor clinic in varie morbos.

A. CORDE. 1. Il pare que le sol valor es prognostic, proque un persistente reduction del nivellos seral de cholinesterase occurreva solmente in patientes qui non superviveva.

2. Illo es sin valor in le manipulation routinari de un patiente con classic infarcimento myocardial si ille responde favorabilemente al therapia.

B. HEPATE. 1. Le nivellos de cholinesterase seral es normal in obstructive morbos del tracto biliari e deprimite in active morbo hepato-cellular. Il pare que illos es plus sensibile in le differentiation de ille typos que le nivellos de transaminase seral.

2. Le nivellos seral de cholinesterase es utile como indicatores del presentia de activitate in morbo parenchymal del hepate.

3. Normal nivellos seral de cholinesterase es provas suggestive del absentia de metastases hepatic in patientes con carcinoma demonstrate in altere partes del corpore.

C. SYSTEMA MUSCULOSKELETIC. 1. Le test del cholinesterase seral es de valor in le differentiation inter dermatomyositis e altere morbos de collageno.

4) In general, in morbos in que le nivellos de transaminase seral es augmentate il occurre un reduction del nivellos de cholinesterase seral. Tamen, quanto al grado e al tempore del alterationes, le disparitate in le curvas pro le duo enzymas indica que il non existe un ver parallelismo in lor comportamento e que il existe nulle relation inter le duo. Le interpretation de un unic determination de cholinesterase seral es semper impossibile a causa del extense variabilitate normal. Mesmo determinationes serial es difficile a interpretar a causa del manco de specificitate. Nonobstante, como supra-indicate, in certe situationes determinationes serial del cholinesterase del sero pote esser de genuin valor clinic.

THE PULMONARY PHYSIOLOGIC DEAD SPACE AS AN INDEX OF EFFECTIVE ALVEOLAR PERFUSION*

By Russell H. Wilson, M.D., Ph.D.
CLINICAL ASSOCIATE PROFESSOR OF INTERNAL MEDICINE, THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL; DIRECTOR OF RESEARCH, VETERANS ADMINISTRATION HOSPITAL

Bruce E. Jay, B.S.
RESEARCH BIOCHEMIST, VETERANS ADMINISTRATION HOSPITAL

Robert S. Meador, M.D.
CLINICAL INSTRUCTOR IN INTERNAL MEDICINE, THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL; ASSISTANT CHIEF CHEST DISEASE SERVICE, VETERANS ADMINISTRATION HOSPITAL

AND

Robert Evans, Ph.D.†

(From the Department of Medicine, The University of Texas Southwestern Medical School, Dallas, Texas, and the Veterans Administration Hospital, McKinney, Texas)

Pulmonary physiologic tests have become widely used by the physician and investigator alike in recent years, although it is often difficult to find a close correlation between a single physiologic test value and the clinical status of the patient. It has become evident that a battery of tests is essential for complete evaluation of the interrelations of ventilation, diffusion, circulation and the anatomic structures which compose the functions and anatomy of the respiratory system.

It is essential for diagnosis and therapy to establish the relationship of the respiratory dead space to the pathologic physiology of patients with pulmonary disease. This is especially true during and following thoracic surgery since there are profound circulatory and ventilatory changes as a result of these procedures (Stead and Soucheray[9]).

By definition, there is an anatomic and physiologic pulmonary dead space volume (Comroe, Forster and Dubois[3]). The anatomic dead space is the volume of the airway conducting the respiratory gases from the nose and mouth to the alveoli. The physiologic dead space, includes the anatomic dead space and the volume of inspired gas ventilating alveoli having inadequate pulmonary capillary blood flow with respect to ventilation (Comroe, Forster and Dubois[3]). There is reason to redefine the latter relationship. In this respect, it is feasible to speculate that in the allegedly hyperventilated alveoli, the deficiency is primarily circulatory since the characteristics of ventilation are qualitatively the same but quantitatively different in the normal and abnormal lung. Nevertheless, an accurately measured pulmonary physio-

*This investigation was supported in part by Research Grants (PHS Grant No. H–2153[C] and No. RG–4258[C2]) from the National Institutes of Health, Public Health Service.

†Mathematical Consultant, Department of Physiology, University of Minnesota, and V.A. Hospital, McKinney, Texas.

TABLE 1.—ANATOMIC AND PHYSIOLOGIC DEAD SPACE VOLUMES FOR RESTING AND EXERCISE PERIODS IN 25 PATIENTS

Patient	*Age*	*Body Surface Area Sq. M*	$P_A CO_2$ *Resting mm. Hg*	$P_A CO_2$ *Exercise mm. Hg*	$P_E CO_2$ *Resting mm. Hg*	$P_E CO_2$ *Exercise mm. Hg*	*Tidal Volume Resting ml.*	*Tidal Volume Exercise ml.*
1. P	67	1.55	39.6	58.5	20.2	25.7	444.8	706.9
2. AGC	60	1.97	40.8	44.9	21.3	23.6	600.4	1088.0
3. McL	56	1.92	41.6	37.2	24.2	29.2	786.8	1136.4
4. JMD	60	1.81	48.1	40.2	21.5	23.3	794.2	1066.0
5. OHF	56	1.84	38.4	35.7	20.7	21.8	691.7	879.9
6. NE	61	1.67	41.8	53.6	18.2	16.4	556.9	937.0
7. HB	61	1.82	35.3	47.8	22.3	26.9	639.9	1294.4
8. LN	59	1.68	41.4	41.0	19.4	26.0	652.4	1049.5
9. AC	62	1.56	50.6	42.1	17.4	22.4	321.7	598.5
10. TJC	57	1.90	21.1	18.8	11.7	14.3	2034.8	3350.5
11. WAS	60	1.84	38.5	41.9	22.6	24.2	828.4	1417.4
12. PA	59	1.82	39.4	47.9	19.9	26.0	647.1	1098.0
13. AHT	62	1.60	36.4	45.5	17.2	23.5	1139.2	1100.3
14. QP	67	1.96	38.3	43.1	20.0	22.6	635.9	916.5
15. WBMcN	63	1.92	38.8	40.3	17.3	17.0	531.8	905.3
16. JC	62	1.67	46.3	50.0	17.6	22.8	604.8	1045.2
17. SF	60	1.76	44.3	36.8	15.1	22.7	602.0	777.8
18. EM	65	1.64	49.9	48.0	22.2	23.4	479.8	630.5
19. RLH	37	1.80	38.5	41.5	24.9	27.7	597.8	901.8
20. WHM	56	1.87	36.8	41.6	19.8	27.7	540.6	1106.9
21. SWR	48	1.83	39.7	48.9	24.3	31.7	419.9	753.0
22. LMcI	61	1.75	40.0	48.6	20.9	26.7	543.2	1081.0
23. SV	61	1.39	38.2	42.3	17.0	22.9	553.7	633.7
24. JEO	33	2.03	35.0	42.7	21.4	17.9	1066.1	1069.6
25. AR	38	2.06	41.5	50.3	27.5	32.8	630.0	1018.3
Averages (25)	57.2	1.79	40.0	43.6	20.2	24.0	693.8	1062.5

logic dead space gives an insight to the perfusion capacity of the lung.

Anatomic and physiologic dead spaces can be measured simply in the clinical physiology laboratory, making available this respiratory parameter to evaluate the patient's pulmonary physiologic status.

In a previous report (Wilson *et al.*[11]), a new method was presented for calculating anatomic dead space. The anatomic dead space was measured in normal persons simultaneously with the physiologic dead space with respect to age and lung volume. Subsequently, the study has been extended to patients with pulmonary disease. We shall be concerned with firstly, the method, secondly, mechanical aids for making the calculation, and lastly, the data (with the interpretation) from the patients with pulmonary emphysema.

In the first report (Wilson *et al.*[11]), reference was made to previous investigators who had reported data pertaining to pulmonary anatomic and physiologic dead space.

Subjects and Procedures. The age range, body surface and number of patients with chronic diffuse pulmonary emphysema studied are listed in Table 1.

The patients and normal persons sat on an exerciser in front of the nitrogen meter, breathing room air through a mouthpiece attached to a system of valves for directing the air flow in one direction. The system of valves was perfused with oxygen to remove nitrogen. At the termination of a normal expiration by the patient, a 5-way valve was turned and the subject inspired 100% oxygen. Upon completion of a maximal inspiration, he expired slowly, uniformly and maximally past the nitrogen meter leak 1 cm. from the mouth. The nitrogen meter curves were analyzed for the inflection point as shown in Fig. 1 with the simple slope calculator shown in Fig. 2. The first and second

TABLE 1.—(*Continued*)

Phys. Dead Space Resting ml.	*Phys. Dead Space Exercise ml.*	*Anat. Dead Space Resting ml.*	*Anat. Dead Space Exercise ml.*	*Timed Vital Capacity 0.5 Sec. ml.*	*Min. Vol. Resting Liters*	*Min. Vol. Exercise Liters*	*Physiologic Dead Space / Tidal Volume %* Resting	*Physiologic Dead Space / Tidal Volume %* Exercise
217.4	396.5	172.2	178.3	591	7.76	14.00	49	56
286.2	516.3	164.4	194.1	1340	12.72	26.55	48	47
328.0	242.5	179.6	167.3	—	12.02	21.94	42	21
438.3	448.0	170.5	155.0	1270	14.16	35.32	55	42
318.5	341.3	174.5	155.2	2010	9.93	4.11	46	39
314.8	650.1	164.2	206.3	324	12.39	31.86	57	69
236.0	564.4	191.8	232.9	2170	8.21	27.01	37	43
346.7	383.9	316.5	220.7	2040	10.39	18.85	53	37
211.0	280.3	150.6	140.0	744	8.41	19.45	66	47
903.5	789.1	308.3	257.2	—	22.61	51.59	44	24
342.3	598.7	164.4	172.9	1620	9.94	29.06	41	42
320.3	501.1	254.6	278.6	1680	9.37	26.86	47	46
601.1	532.9	210.8	177.0	591	17.99	19.64	52	48
303.1	434.9	133.5	173.5	634	12.72	26.12	48	47
294.8	524.1	189.7	223.2	2220	13.19	28.77	55	58
374.5	568.2	155.0	171.0	1360	10.38	22.98	62	54
397.1	297.4	185.5	118.6	818	9.70	28.66	66	38
266.6	323.1	252.1	206.4	378	9.49	19.00	55	51
215.8	299.9	215.4	316.1	—	9.88	28.14	36	33
250.4	369.8	185.0	208.5	2330	9.05	26.65	46	28
163.0	265.0	126.5	180.8	914	9.97	17.57	38	35
259.2	487.1	155.1	150.2	648	13.19	28.47	48	45
307.5	291.2	258.0	229.4	—	14.15	25.03	55	46
414.8	619.6	358.1	226.5	585	16.89	32.09	39	58
212.6	353.2	248.0	266.0	2156	9.70	18.33	34	34
332.9	443.1	203.4	200.2				49	44

derivatives of any curve can be easily calculated with meticulous use of these simple devices*. Calculation of reaction rates, dye curve characteristics, and velocities of flow are readily made by persons who are not primarily mathematicians. The details, proof and development of this method are outlined in a previous publication (Wilson *et al.*[11]). Briefly, the principle is to find the inflection point of the nitrogen meter curve by taking its first and second derivatives with respect to time, and project it onto the pneumotachograph volume flow curve to calculate the anatomic dead space (Fig. 1).

The alveolar carbon dioxide tension was assumed to be the same as that of the arterial blood. It was measured by the method of Riley[7].

The physiologic dead space was measured by the method of Bohr[1], simultaneously with the anatomic dead space volume during rest and following a 5-minute period of vigorous exercise.

A Waters Conley nitrogen meter was used to measure the rate of change of nitrogen in a single expired breath. The nitrogen meter circuits were modified** to decrease its 90% response time to less than 0.03 second.

The output of the nitrogen meter was led into an Electronics for Medicine Physiologic Recorder†. Volume flow was measured with a pneumotachograph which was calibrated daily with air. There was no significance of difference between air and oxygen calibrations.

Results. The anatomic and physiologic dead space volumes for the patients are listed for resting and exercise periods in Table 1.

The anatomic dead space in the pa-

*Machined by Mr. John Ognanovich, Veterans Administration Hospital, Minneapolis, Minnesota.

**Mr. R. A. Huffhines, Dallas Radionics, Dallas, Texas.

†Electronics for Medicine, 17 S. Lexington Ave., White Plains, New York.

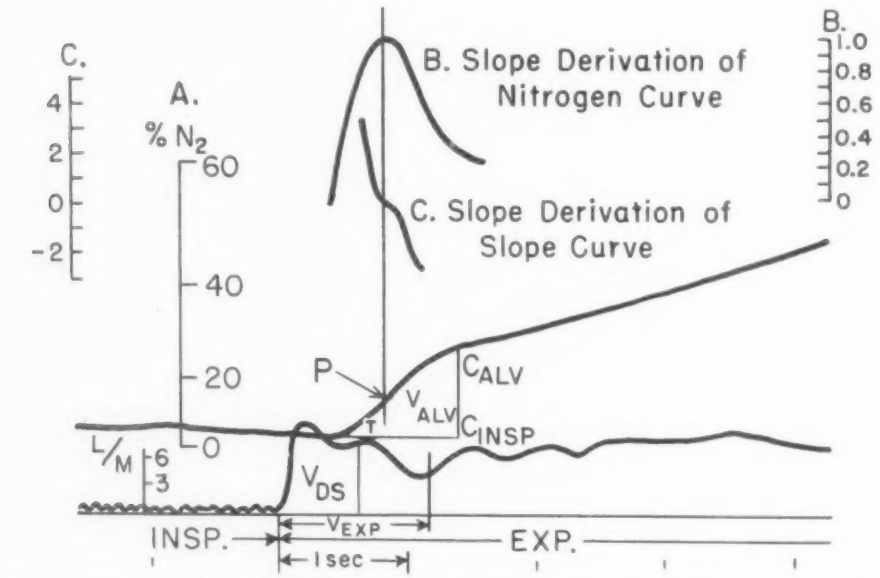

Fig. 1.—Analysis of the single breath curve of the nitrogen meter for calculating anatomic dead space. *A*, scale for the nitrogen meter curve; *B*, the first derivative; *C*, the second derivative; *P*, the Inflection point; *L/M* scale for pneumotachographic curve; Data for Bohr Formula: V_{EXP}, Volume of Expired Air; V_{DS}, Volume of Dead Space; C_{ALV}, Concentration of N_2 in alveolar air; V_{ALV}, Volume of expired gas; C_{INSP}, Concentration of same gas in inspired air.

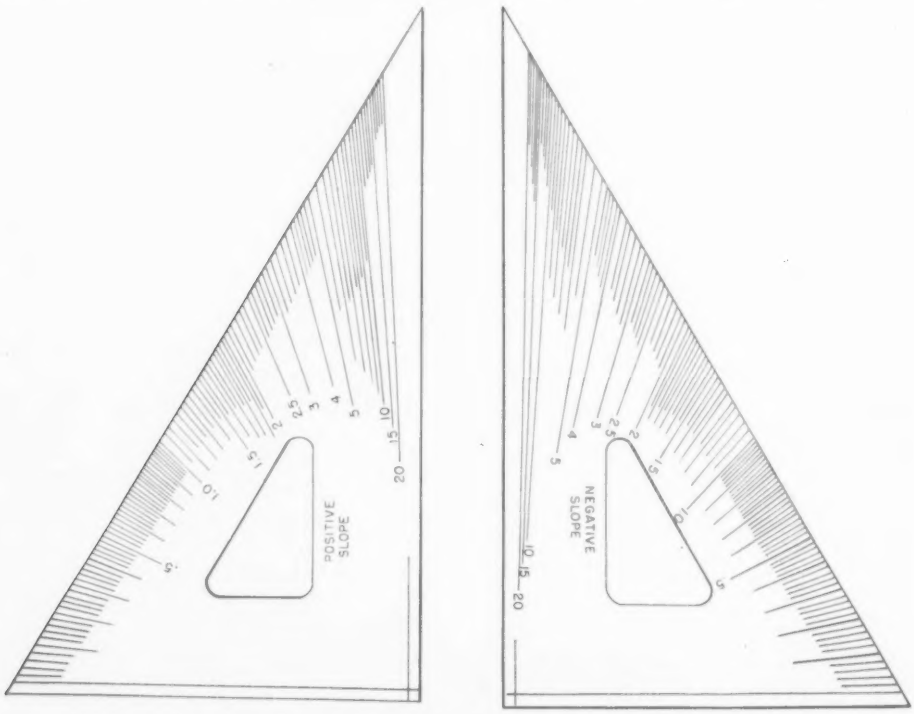

Fig. 2.—The Positive and Negative Slope Calculators.

tients changed from a mean value of 203 ml. during rest to 200 ml. during exercise. This was of no significance ($P>0.8$). The volume of the anatomic dead space in the normal persons of a comparable age increased from 180 ml. at rest to 191 ml. during exercise $0.3<P<0.4$.

On the other hand, the magnitude of increase in physiologic dead space was large in the patients changing from a primarily increased value of 333 ml. during rest to 443 ml. during exercise ($P<0.01$). In contrast in persons with normal lungs, the physiologic dead space decreased during exercise from a mean value of 307 ml. to 245 ml. $0.1<P<0.2$. This indicates improvement of the ventilation perfusion relationship during exercise. The increased pulmonary blood flow and cardiac output contribute to this phenomenon.

There was no correlation between the 0.5 second expiratory capacity and the physiologic dead space $P>0.3$, although the patients with severe emphysema had the largest physiologic dead space and the smallest 0.5 second vital capacity.

The P_A CO_2 in the patients with emphysema increased from an average of 40.0 at rest to 43.6 mm. Hg during exercise. The P_E CO_2 increased from 20.2 at rest to 24.0 during exercise.

The mean patient ratio of the physiologic dead space to the tidal volume at rest was 49% and decreased to 44% after exercise. Each was significantly above the normal value as determined by Riley[6].

Discussion. The mean volume of the anatomic dead space, at a given lung volume was not further significantly increased in the patients with emphysema during exercise, but indeed, showed a highly significant resting increase when compared with that of a normal group of comparable age (Wilson *et al.*[11]). The volume of the anatomic dead space was found to increase with inflation of the lung in normal persons (Wilson *et al.*[11]); however, since the lungs in patients with severe emphysema are in a continuous state of hyperinflation with an increase in the volume of the anatomic dead space at rest, there was no increase of the dead space volume with hyperventilation during exercise.

The pulmonary alveoli are ventilated by a combination of simple diffusion of oxygen in, carbon dioxide out, and by air flow, thus, an increase in length and tortuosity of the bronchioles would decrease diffusional and ventilatory efficiency. These respiratory impediments in the emphysematous lungs superimposed on the change of the elastic properties (Stead, Fry and Ebert[8]) of the lungs lead to irreversible hypoxia and retention of carbon dioxide.

In a group of patients with severe pulmonary emphysema whose oxygen saturation of the blood was low initially, there was no significant change in this value during hyperventilation (Wilson *et al.*[10]). The combination of abnormal retention of carbon dioxide and inability to increase the per cent saturation of the hemoglobin with oxygen during hyperventilation is consistent with decreased perfusion of the normally ventilated alveoli. The number of well ventilated and perfused alveoli is inadequate to lower the P_A CO_2 during hyperventilation. By the same token, because each gram of normal hemoglobin can combine chemically with a maximum of 1.34 ml. of oxygen, the number of alveoli perfused with normal quantities of blood is insufficient to increase the per cent oxygen saturation of the hemoglobin in the presence of severe pulmonary emphysema, regardless of patient's in-

creased respiratory effort and increased cardiac output.

An increase of the physiologic dead space during rest and exercise indicates nothing more than the concept that portions of the lung are hyperventilated without a concurrent increase in perfusion. Exercise with the increased respiratory minute volume merely caused further divergence of the alveolar ventilation-perfusion relationship from normal.

The concept of the ratio of alveolar ventilation to alveolar perfusion with capillary blood has not been utilized extensively in the evaluation of pulmonary pathologic physiology because of the technical intricacies encountered in quantitating the factors composing the ventilation perfusion ratio. This is no longer a problem with the advent of the nitrogen meter. The pulmonary physiologic dead space as defined by Comroe[3] is an indirect measurement of the alveoli ventilated with oxygen in excess of the quantity of perfusing erythrocytic hemoglobin. A large value for the physiologic dead space indicates first, inadequate perfusion of the well ventilated areas of the lung. In the presence of a large pulmonary physiologic dead space, emphysematous blebs, fibrosis, and infiltration, one can assume and expect diminished effective alveolar perfusion because of a smaller number of capillaries as shown in the anatomic studies of Isaakassohn[4].

A second factor which greatly influences the alveolar-capillary diffusion gradient for oxygen and carbon dioxide is the rate of flow of blood through a pathologic capillary bed (Luchsinger, McCormick and Moser[5]). In pulmonary emphysema, there is evidence to indicate pathologic reduction of the volume of the capillary vascular bed which could explain the marked increase in the physiologic dead space during exercise in the patients. The exercise increased the alveolar ventilation without a significant increase in the volume of the pulmonary capillary vessels. Further, since the cardiac output is normal or increased at rest in patients with emphysema and hypoxia (Borden *et al.*[2]), one can infer a greater velocity of the blood flowing through the pulmonary capillaries which prevents optimal contact of the erythrocytes with the alveolar oxygen.

Summary. The anatomic and physiologic dead space was measured in 25 patients with moderate to severe emphysema.

The mean resting physiologic dead space of 333 ml. was increased to 443 ml. during exercise. The mean resting anatomic dead space of 203 ml. decreased to 200 ml. during exercise.

A large physiologic dead space indicates extensive diminution of the volume of the alveolar vascular bed with an increased velocity in the blood flow through the pulmonary capillaries in the presence of a normal cardiac output.

The ratio of physiologic dead space to tidal volume was increased to 49% at rest and 44% during exercise.

REFERENCES

1. **Bohr, C.:** Skandinav. arch. f. Physiol., **2**, 236, 1890.
2. **Borden, C. W., Wilson, R. H., Ebert, R. V.,** and **Wells, H. S.:** Am. J. Med., **8**, 701, 1950.
3. **Comroe, J. H., Forster, R. E.,** and **Dubois, A. B.:** Pulmonary Ventilation. The Lung. Chapt. 3, 21, 1955. Chicago: The Year Book Publishers, Inc., 1955.
4. **Isaakassohn, H. von:** Virchows Arch. f. Path. Anat., **13**, 466, 1871.
5. **Luchsinger, P. C., McCormick, G. S.,** and **Moser, K. M.:** Clin. Res. Proc., **5**, 118, 1957.

6. **Riley, R. L.,** and **Cournand, A.:** J. Appl. Physiol., **1,** 825, 1949.
7. **Riley, R. L., Proemmel, D. P.,** and **Franke, R. E.:** J. Biol. Chem., **161,** 621, 1945.
8. **Stead, W. W., Fry, D. L.,** and **Ebert, R. V.:** J. Lab. & Clin. Med., **40,** 674, 1952.
9. **Stead, W. W.,** and **Soucheray, P. H.:** J. Thoracic Surg., **23,** 453, 1952.
10. **Wilson, R. H., Borden, C. W., Ebert, R. V.,** and **Wells, H. S.:** J. Lab. & Clin. Med., **36,** 119, 1950.
11. **Wilson, R. H., Jay, B. E., Richburg, P. L.,** and **Evans, R.:** Am. J. Med. Sci., **232,** 67, 1956.

SUMMARIO IN INTERLINGUA

Le Physiologic Spatio Morte Pulmonar Como Indice de Efficace Perfusion Alveolar

Le spatios morte anatomic e physiologic esseva mesurate in 25 patientes con emphysema de grados moderate a sever.

Le valor medie de 333 ml pro le spatio morte physiologic in stato de reposo esseva augmentate a 443 ml durante exercitio. Le valor medie de 203 ml pro le spatio morte anatomic in stato de reposo descendeva a 200 ml durante exercitio.

Un grande spatio morte physiologic indica un diminution extense del volumine del vasculatura alveolar con augmento del velocitate del fluxo sanguinee a transverso le capillares pulmonar in le presentia de un normal rendimento cardiac.

Le proportion inter spatio morte physiologic e volumine de aere currente se monstrava augmentate a 49 pro cento in stato de reposo e a 44 pro cento durante exercitio.

SUMMARIO IN INTERLINGUA

(See page 554 for original article)

Un Evalutation de Necropsias a Agulia

Es reportate le constatationes obtenite per necropsia a agulia in 63 casos. In 50 de illos, le constatationes esseva verificate per necropsias complete.

Necropsia a agulia es de valor quando le permisso pro un necropsia complete non pote esser obtenite. (Tamen, permissos in forma scribite deberea esser obtenite etiam pro necropsias a agulia.) Le comparation del resultatos obtenite in le mesme patiente per necropsia a agulia e per necropsia complete revelava discrepantias in 52% del casos. Isto illustra le limitationes del technica de necropsia a agulia. Diagnoses inadequate pote resultar proque le organo o le sito interessate non es accessibile al agulia o proque un specimen ab iste areas non es representative pro le organo o systema integre. In plus, le specimen obtenite pote esser inadequate in consequentia del facto que le technica exclude visualisation directe.

Morbos que affice organos como le hepate, le renes, e le pulmones e que produce massas palpabile in ille organos se presta plus facilemente al diagnose per necropsia a agulia. In morbos de iste organos, le diagnose correcte esseva obtenite per medio de agulias in omne casos del presente serie in que le specimens esseva adequate.

A parte le facto que necropsia a agulia se presta al verification de un impression clinic, ille etiam provide a vices un diagnose que non se suspiceva super le base de observationes clinic.

AN EVALUATION OF NEEDLE NECROPSIES

By Michael West, M.D.
RESIDENT, MEDICAL SERVICE, WEST SIDE VETERANS ADMINISTRATION HOSPITAL

AND

Bernhard Chomet, M.D.
CHIEF, LABORATORY SERVICE, WEST SIDE VETERANS ADMINISTRATION HOSPITAL; ASSISTANT PROFESSOR OF PATHOLOGY, THE CHICAGO MEDICAL SCHOOL

(From the Medical and Laboratory Services, West Side Veterans Administration Hospital, Chicago, Illinois)

Frequently the efforts of a physician to obtain permission for a necropsy are thwarted by widespread prejudice against this procedure. As recently as 1955, postmortem examinations were performed in only one-third of all hospital deaths in the United States[3]. In such situations, when consent for a complete necropsy is refused, the suggestion of a "needle necropsy" as first described by Terry[6], may be less reluctantly accepted by the relatives. By this technique postmortem tissue specimens from various sites were obtained by the Vim-Silverman needle. He found this method to be helpful and in 22 of 24 needle necropsies this procedure led to a diagnosis.

This communication deals with 50 consecutive necropsies performed in the usual manner after tissue specimens had been obtained by the needle necropsy technique. The results of both methods will be compared.

Needle necropsies were also performed in 13 other cases when permission for a complete necropsy was not obtained.

Materials and Methods. The instrument employed in this study was a large Vim-Silverman needle[5]. The technique in the actual "biopsy" was that used for percutaneous needle biopsy of the liver or kidney. Aspiration with a syringe was not performed. By utilizing one puncture site multiple specimens of one organ were obtained by changing the direction of the needle.

Multiple samplings of the liver, lungs and kidneys were attempted in each case. Attempts at obtaining tissue from other sites were determined by the clinical findings. The heart and brain were not "biopsied" since it was not expected that specimens from these organs would be sufficiently representative to contribute to the diagnosis. Nevertheless, it is conceivable that such heart diseases as diffuse myocarditis or amyloidosis and localized brain lesions as a tumor might be diagnosed by this method. In addition to the liver, lungs and kidneys, other organs were sampled when indicated as were palpable masses of undetermined origin. In the presence of pleural effusion, specimens were obtained from the pleura (Heller, Kellow and Chomet[2]). Body fluids such as pericardial, pleural, ascitic fluid, cerebrospinal fluid and heart blood were obtained for cell block studies or culture, or both.

The prostate was sampled on several occasions. The following procedure was used: The prostate was palpated through the rectum and the needle was introduced in the midline of the lower abdomen just above the pubic symphysis. A specimen was taken when the needle tip was felt by the finger palpating the prostate. Specimens were obtained from the pancreas in a similar manner as from the kidneys. Those from the kidney were obtained from a site which was approximately 1 cm. below the twelfth rib and 2 cm. lateral to the lateral border of the quadratus lumborum muscle, while the tissue from the pancreas was obtained from a site 2 cm. medial to the lateral border of this muscle and 1 cm. below the twelfth rib.

The specimens obtained were 0.1 cm. in diameter and varied in length, measuring up

to 2 cm. The tissue from one necropsy was placed in a jar with 10% formalin, and then was embedded in paraffin, usually in one block, and serial sections were taken. The sections were stained with hematoxylin and eosin. Figure 1 represents the gross appearance of a typical slide with all the tissue from a needle necropsy.

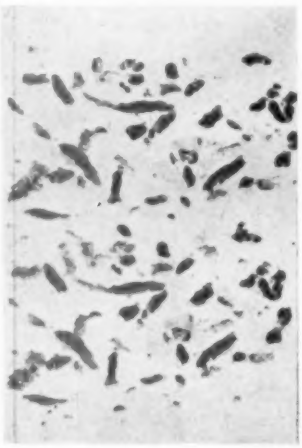

Fig. 1.—A typical slide with tissue from one needle necropsy. × 2.5.

The needle necropsy specimens were examined without knowledge of the clinical findings or anatomical diagnosis of the complete necropsy. The organs from which the specimens were obtained were diagnosed with ease except on three occasions when only malignant tissue was seen. Demonstration of malignant tissue, even if the primary site was not identified, was considered adequate for a diagnosis.

Results. In the 13 patients in which only needle necropsies were performed, a diagnosis was made in 10 or 77% (Table 1).

In 50 patients both needle and complete necropsies were performed (Table 2). In 24 patients (48%), the diagnosis was the same by both techniques. There was no agreement in 26 patients (52%). Of the 24 patients in which the needle necropsy diagnoses were adequate, 17 were made from liver specimens, 4 from kidney specimens and 3 from lung specimens.

In the 26 patients in whom the needle necropsy did not lead to the diagnosis from which the patient died, there were 18 who had such diseases as myocardial infarction, pulmonary infarction, cerebral vascular accidents, heart failure, gastrointestinal hemorrhage, peritonitis and enterocolitis. There were 2 patients who had died in diabetic coma. In the other 6 patients, inadequate specimens were obtained from the critical sites.

Occasionally the clinical impression is not substantiated by the necropsy findings (Hunter[4]). The needle necropsies also revealed unsuspected diseases on several occasions, as illustrated by the following 2 case reports.

TABLE 1.—RESULTS OF THE NEEDLE NECROPSIES IN THE GROUP OF 13 CASES WITHOUT ROUTINE NECROPSIES

1. Chronic lymphatic leukemia of the spleen, liver and lymph nodes.
2. Laennec's cirrhosis. Bile nephrosis.
3. Metastatic carcinoma of the liver and left upper quadrant mass. (primary pancreas?).
4. Metastatic small-cell carcinoma to the liver.
5. Squamous-cell carcinoma of the lung, liver and cervical node.
6. Laennec's cirrhosis.
7. Chronic pyelonephritis.
8. Adenocarcinoma of the lung and liver.
9. Adenocarcinoma of the stomach with metastases to the lung, pancreas, liver and lymph nodes.
10. Small-cell carcinoma (pleura).

11.–13. No adequate pathological diagnosis.

TABLE 2.—RESULTS OF THE NEEDLE NECROPSIES PERFORMED IN CONJUNCTION WITH COMPLETE NECROPSIES

Group A. Adequate Diagnosis Made by Needle Necropsy

Needle Necropsy Diagnosis:	*Complete Necropsy Diagnosis:*
1. Laennec's cirrhosis	1. Laennec's cirrhosis
2. Lymphoma of liver with area of necrosis	2. Reticulum cell sarcoma of nodes, liver and spleen
3. a) Metastatic adenocarcinoma to liver b) Pyelonephritis of kidneys	3. a) Adenocarcinoma of prostate b) Metastases to the liver, lungs, spleen, and adrenals c) Pyelonephritis of kidneys
4. Small-cell carcinoma of lung with metastases to the liver	4. Small-cell carcinoma of lung with metastases to the hilar nodes, liver and adrenals
5. Chronic pyelonephritis	5. Chronic pyelonephritis
6. a) Metastatic adenocarcinoma to liver b) Central necrosis and bile stasis of liver	6. a) Adenocarcinoma of head of pancreas, with metastases to liver b) Obstructive jaundice
7. Malignant nephrosclerosis	7. Malignant nephrosclerosis
8. Metastatic small-cell carcinoma to liver	8. a) Small-cell carcinoma of lung b) Metastases to liver
9. Metastatic adenocarcinoma to liver	9. a) Adenocarcinoma of stomach b) Metastases to gallbladder and liver
10. a) Metastatic squamous-cell carcinoma to liver	10. a) Squamous-cell carcinoma of bladder with metastases to liver b) Chronic pyelonephritis
11. a) Metastatic small-cell carcinoma to liver b) Radiation pneumonitis c) Acute hepatic necrosis	11. a) Bronchogenic carcinoma b) Metastases to liver c) Radiation pneumonitis d) Acute hepatic necrosis
12. Fatty cirrhosis	12. Fatty cirrhosis
13. Laennec's cirrhosis	13. Laennec's cirrhosis
14. Fatty cirrhosis	14. Fatty cirrhosis
15. Metastatic adenocarcinoma to liver	15. a) Adenocarcinoma of pancreas b) Metastases to lymph nodes and liver c) Acute endocarditis with emboli to kidney and spleen
16. a) Metastatic squamous-cell carcinoma to liver b) Bronchopneumonia	16. a) Small-cell carcinoma of lung b) Metastases to liver, adrenals c) Bronchopneumonia
17. Early fatty cirrhosis	17. a) Early fatty cirrhosis b) Wernicke's disease
18. a) Acute hepatic necrosis b) Bile nephrosis	18. a) Acute hepatic necrosis b) Bile nephrosis
19. a) Intercapillary glomerulosclerosis	19. a) Acute leukemia (clinical) b) Intercapillary glomerulosclerosis
20. Fatty cirrhosis	20. Fatty cirrhosis
21. Undifferentiated carcinoma of lung	21. Anaplastic alveolar cell carcinoma of lung
22. Small-cell carcinoma of lung	22. a) Small-cell carcinoma of the lung with metastases to liver and nodes b) Empyema of gallbladder
23. Metastatic adenocarcinoma of liver	23. a) Carcinoma of prostate b) Metastases to liver, nodes, vertebrae and kidneys
24. Necrotizing angiitis of kidney	24. Periarteritis nodosa

TABLE 2.—RESULTS OF THE NEEDLE NECROPSIES PERFORMED IN CONJUNCTION WITH COMPLETE NECROPSIES *(Continued)*

Group B. Inadequate Diagnosis Made by Needle Necropsy

Needle Necropsy Diagnosis:	*Complete Necropsy Diagnosis:*
1. Hyperemia of liver and kidney	1. Ruptured cerebral aneurysm
2. Hyperemia of liver	2. a) Old myocardial infarction b) Bilateral pneumonitis
3. Hyperemia and fatty metamorphosis of liver	3. Pulmonary emboli
4. Central necrosis of liver	4. Peptic ulcer with hemorrhage
5. a) Pneumonia b) Hyperemia of kidneys	5. Hemorrhage of brain
6. Chronic passive hyperemia of liver, lung and kidneys	6. Pseudomembranous enterocolitis
7. Hyperemia of liver	7. Scirrhous carcinoma of stomach
8. Hyperemia of liver and lungs	8. Myocardial infarction
9. Hyperemia of kidney and liver	9. a) Idiopathic cardiac hypertrophy b) Multiple infarcts of lungs and kidneys
10. a) Hyperemia of liver and lungs b) Focal necrosis of pancreas c) Benign nephrosclerosis	10. a) Sclerosing coronary artery disease b) Encephalomalacia c) Focal necrotizing pancreatitis d) Pulmonary embolus
11. a) Chronic pyelonephritis b) Fatty liver	11. a) Carcinoma of the prostate with metastases to vertebrae and adrenals b) Chronic pyelonephritis
12. a) Hyperemia of lungs b) Portal fibrosis of liver (no kidney specimen)	12. a) Chronic glomerulonephritis superimposed on chronic pyelonephritis
13. Postnecrotic cirrhosis	13. a) Hepatoma b) Postnecrotic cirrhosis
14. a) Fatty liver b) Bronchopneumonia	14. a) Diabetic coma (clinical) b) Fatty liver and bronchopneumonia c) Chronic pancreatitis
15. a) Hyperemia of liver	15. a) Carcinoma of rectum with perforation and peritonitis
16. Hyperemia of lung and liver	16. a) Myocardial infarction b) Pulmonary embolus
17. Chronic passive congestion of liver and lungs	17. Rheumatic heart disease
18. Benign nephrosclerosis	18. Ruptured aortic aneurysm
19. Hemosiderosis of liver with focal necrosis and fibrosis	19. a) Aplastic anemia b) Transfusion hemosiderosis with focal necrosis of liver c) Bronchopneumonia
20. a) Central necrosis of liver b) Hyperemia of kidneys	20. a) Cor pulmonale b) Pulmonary emphysema c) Bronchiectasis
21. Hyperemia of liver and lungs	21. a) Hemangiosarcoma of stomach, heart, lungs, and brain
22. Central necrosis of liver	22. a) Old and recent myocardial infarction b) Central necrosis of liver
23. Hyperemia of liver	23. a) Myasthenia gravis b) Thymoma
24. a) Central necrosis of liver b) Nephrosclerosis, benign	24. a) Pulmonary infarction b) Old myocardial infarction c) Benign nephrosclerosis
25. Fatty metamorphosis of liver	25. a) Diabetic coma b) Fatty liver
26. a) Chronic pyelonephritis b) Fatty liver	26. a) Carcinoma of prostate, metastases to bone b) Chronic pyelonephritis

Fig. 2.—Stomach with normal pyloric mucosa and areas of mucus-producing adenocarcinoma. × 80.

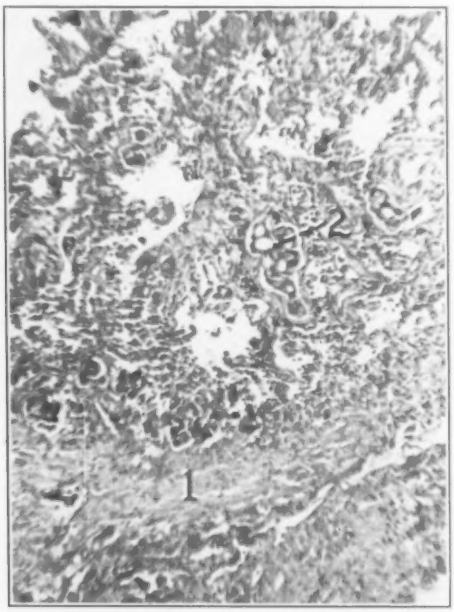

Fig. 3.—Section from lung showing pleura (1) and vascular invasion by carcinoma (2). × 80.

Case Reports. CASE 1. A 46-year-old man came to the hospital because of weakness and exertional dyspnea of recent onset. Except for marked pallor, the physical examination was not remarkable and extensive laboratory work-up revealed the following pertinent data. The hemoglobin was 5 gm.%, liver functions were normal except for an alkaline phosphatase of 197 Bodansky units (upper normal 4 units). The acid phosphatase was 5.9 King-Armstrong units (upper normal 2.0 units) and the prostatic acid phosphatase was 2.8 units (upper normal 0.6 units) (Fishman and Lerner[1]). A chest roentgenogram revealed miliary nodules in both lung fields. Roentgenograms of the bones were not remarkable. A complete gastrointestinal series was normal, although the stomach appeared small and peristalsis appeared to be greatly decreased in this organ during fluoroscopy. The bone marrow contained groups of anaplastic carcinoma cells. The patient died after a brief, fulminant course. Permission for a routine necropsy was refused. However, because the primary site of the carcinoma was not determined and because of the unexplained extremely high alkaline phosphatase and the elevated acid and prostatic acid phosphatase, permission for a needle necropsy was requested and granted. The clinical story was reviewed and the following sites were considered critical: the lungs, stomach, and prostate. Actually, adequate specimens were obtained from the following sites: stomach, liver, lungs, kidneys, prostate, pancreas, and a hilar lymph node. The section of stomach presented the picture of a mucus-producing adenocarcinoma (Fig. 2). There was histological evidence of invasion of the pancreas with metastases to the lungs (Fig. 3), liver and hilar lymph node. Multiple specimens of the prostate failed to reveal carcinoma.

CASE 2. A 79-year-old man came to the hospital with complaints of fever, weakness, dyspnea, cough and hemoptysis of one month's duration. He also complained of "aching" pain in the legs. The patient appeared to be chronically ill; he was pale, dehydrated and moderately confused. His blood pressure was 188/100 and his heart rate was 110 per minute. No other gross abnormal findings were described. The hemoglobin was 8 gm. % and the nonprotein nitrogen was 207 mg., and creatinine 9.0 mg. The roentgenogram of the chest revealed a small opacity adjacent to the right hilum. The patient became increasingly stuporous and died within a few days; thus there was little opportunity to investigate his obvious renal disease. The clinical picture indicated that this patient may have had a broncho-

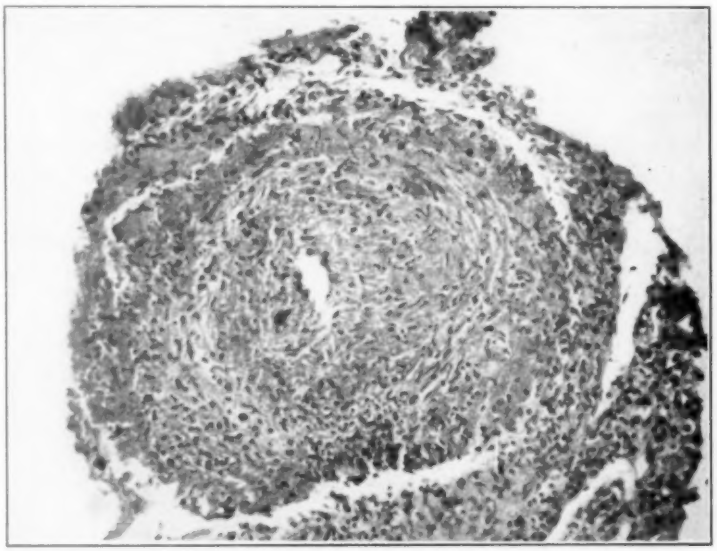

Fig. 4.—Section from kidney with necrotizing angiitis. × 155.

genic carcinoma. Before the needle necropsy was performed it was decided that the kidneys, liver and lungs were the critical sites. Specimens of the kidneys revealed necrotizing angiitis (Fig. 4). No carcinoma was exhibited by the needle necropsy. The diagnosis of the complete necropsy was periarteritis nodosa. No carcinoma was found.

Summary. Reported above are the findings by needle necropsy in 63 cases, of which 50 were checked by complete necropsy.

Needle necropsy is of value when permission for a complete necropsy cannot be obtained. (A written consent, however, should be obtained for the performance of a needle necropsy.) The comparison of the results of needle and complete necropsies of the same patients revealed discrepancies in 52%. This demonstrates the limitations of this procedure. Inadequate diagnoses may result because the involved organ or site is not accessible to the needle or because a specimen from these areas might not be representative of the entire organ or system. Also the sample obtained may be inadequate because this is a "blind" procedure.

Diseases which involve organs like the liver, kidneys, and lungs and which produce palpable masses are more amenable to diagnosis by the needle necropsy technique. In disease of these organs, the correct diagnosis was made in each instance where adequate specimens were obtained.

In addition to substantiation of the clinical impression, the needle necropsy can provide diagnoses which are not suspected clinically.

ACKNOWLEDGMENT: We are grateful for the technical assistance of Miss Olevia Bennett and Mrs. Gaida Barens.

REFERENCES

1. **Fishman, W. H.,** and **Lerner, F. M.:** J. Biol. Chem., **200,** 89, 1953.
2. **Heller, P., Kellow, W. F.,** and **Chomet, B.:** New England J. Med., **255,** 684, 1956.
3. Hospitals Guide Issue: Hospitals, Part II, **30,** 64, August 1, 1956.
4. **Hunter, W. S.:** Permanente Foundation Med. Bull., **10,** 306, 1952.
5. **Silverman, I.:** Am. J. Surg., **40,** 671, 1938.
6. **Terry, R.:** J. Clin. Path. (London), **8,** 38, 1955.

(Interlingua on page 553)

SUMMARIO IN INTERLINGUA

(See page 493 for original article)

Studios Metabolic In Patientes Recipiente Hormon Mobilisator De Lipido

1. Mobilisator de lipido esseva administrate diurnemente durante 2 septimanas a 4 patientes mantenite a un dieta que provideva solmente 4,8 g de grassia per die. In iste individuos, il resultava un elevation frappante del cholesterol del plasma, del acidos grasse, e del phosphoro lipidic e un augmento definite in le excretion fecal e urinari de cholesterol.

2. Iste effecto non esseva observate quando le mobilisator de lipido esseva administrate a 2 altere subjectos mantenite a dietas que provideva 99 e 153 g de grassia per die, respectivemente.

3. Le responsa a mobilisator de lipido depende apparentemente del appropriate conditiones metabolic al tempore del injection. Un dieta que es multo basse in su contento de grassia permitte un marcate hyperlipemia post le administration de mobilisator de lipido, durante que un dieta a alte contento de grassia prevene un tal responsa a simile quantitates de mobilisator de lipido.

PROGRESS
OF
MEDICAL SCIENCE

THERAPEUTICS

UNDER THE CHARGE OF

HARRY GOLD, M.D.
PROFESSOR OF CLINICAL PHARMACOLOGY, CORNELL UNIVERSITY MEDICAL COLLEGE

AND

McKEEN CATTELL, M.D.
PROFESSOR OF PHARMACOLOGY, CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, NEW YORK

IMMUNOLOGICAL ASPECTS OF TOBACCO AND SMOKING*

BY H. SILVETTE, PH.D.

P. S. LARSON, PH.D.

AND

H. B. HAAG, M.D.
DEPARTMENT OF PHARMACOLOGY, MEDICAL COLLEGE OF VIRGINIA
RICHMOND, VIRGINIA

(From the Department of Pharmacology, Medical College of Virginia, Richmond, Virginia)

A SURVEY of the literature has failed to reveal any previous comprehensive review of the immunology of tobacco. [Tobacco, used thus without qualification, may be taken to refer to tobacco itself, or to any preparation of tobacco, including tobacco smoke, or to any constituent of tobacco or tobacco smoke.] The adjective "comprehensive" applied to the present review is in one sense, however, a misnomer: the immunology of tobacco has been far from comprehensively investigated, and the gaps in our knowledge of this subject, more particularly of the fundamental mechanisms involved, will become only too apparent in the course of the discussion, though these may have the desirable effect of directing attention to the urgent need for basic immunological tobacco research on experimental animals. But in another sense, this review is comprehensive, in that it brings into one compass the avail-

*Supported by a grant from the Tobacco Industry Research Committee. We also wish to acknowledge the kind assistance of the Library of the American Tobacco Company Research Laboratory for obtaining for our review many of the articles cited in foreign publications.

able published work, experimental and clinical, treating of immunological aspects of tobacco and smoking.

This review will be divided into 3 parts, the first dealing with the immunology of nicotine, the second with the immunology of tobacco and tobacco smoke, the third with the influence of tobacco on immune reactions.

PART I. IMMUNOLOGY OF NICOTINE. The chemical configuration of the nicotine molecule affords no reason to believe that nicotine is in itself antigenic. A vast number of investigations on the acquired tolerance of nicotine in many animal species have furnished no evidence that injection of nicotine gives rise to any immunological response whatsoever, although it should be pointed out that the technique of drug administration, particularly in its quantitative and temporal aspects, used to investigate the phenomenon of tolerance differs radically from that employed in attempts to elicit antibody formation. It should be further borne in mind that certain simple chemical compounds, not antigenic in themselves, may, as Landsteiner and his co-workers have demonstrated, give rise to antibody formation through combination with body proteins; but the necessary techniques for eliciting this phenomenon do not appear to have been applied to nicotine.

The nonantigenic nature of nicotine is implicit in an early experiment by Hatcher[52], who was unsuccessful in an attempt to use serum of nicotine-tolerant rabbits to protect guinea pigs from the toxic action of nicotine. Bernot[7] stated that it was not possible to obtain even mild immunization against the toxic effects of tobacco smoke in dogs injected daily for 2 to 3 months with 1 mg. nicotine.

Fukuda[33a,b] gave 2 rabbits 1 mg./kg. nicotine subcutaneously twice a day, and reported that the animals showed no allergic reaction to the drug. To 2 other rabbits receiving nicotine, he gave daily intravenous injections of 2 ml./kg. of bovine serum; these animals developed positive Arthus reactions to intradermal injection of the serum, and of the serum plus nicotine, but not to nicotine alone. Thus, nicotine did not here act as a haptene (that is, an incomplete antigen incapable alone of causing the production of antibodies, but capable of neutralizing specific antibodies); but other experiments by Fukuda appear to suggest that nicotine may possibly possess some still-obscure haptenic activity. Following a report by Takatsu and Araki (Nippon J. Angio-Cardiology, 11, 75, 1947) on the presence of an antibody specific for cardiac phosphatide in the blood of subjects suffering from myocardial disturbance, Fukuda[33] studied 7 rabbits given nicotine plus bovine serum and 6 rabbits given nicotine only. Most of the animals given the combination of nicotine and foreign protein exhibited a positive cardiac phosphatide reaction and electrocardiogram, and histologic studies clearly indicated the presence of myocarditis; most of the rabbits given nicotine alone exhibited the reaction, but without evidence of myocarditis.

As a preface to the discussion of tobacco allergy in man, a distinction must be drawn between the immunological and pharmacological effects of nicotine and tobacco. It is well recognized that 2 types of abnormal reaction to these substances may be distinguished, the first an exaggeration of the characteristic pharmacological effects of tobacco or nicotine on a normal person (namely, a *quantitative* difference in response), the second the occurrence of phenomena which do not resemble the ordinary effects of tobacco or nicotine (that is, a *qualitative* difference in response), but be-

long to the allergic type (that is, antigen-antibody reaction). There has been, however, a considerable amount of confusion with respect to terminology, the terms "idiosyncrasy" and "hypersensitivity" being employed, now for one, now for the other, of the 2 types of responses, and occasionally even being used as synonyms. In this review, *idiosyncrasy* will signify that *qualitative* response to tobacco or nicotine which does not resemble the ordinary pharmacological effects of these substances, but constitutes an allergic reaction, while *hypersensitiveness* or *hypersensitivity* refers to the *quantitative* difference in response to the ordinary pharmacological actions. It will be seen that idiosyncrasy bears the same relationship to immunity as hypersensitiveness to tolerance.

A very curious report by Karrenberg[61], if taken at its face value, appears to indicate that nicotine may act as a haptene. Karrenberg's patient showed strong patch tests to tobacco alone; and 1 drop of 1:1 million nicotine prepared from crude tobaccos massaged gently into an area of the skin which had been superficially scraped resulted in characteristic symptoms of anaphylactic shock. Although the probability is that the nicotine solution so prepared also contained traces of protein anaphylactogenic material, the occurrence of anaphylaxis is noteworthy.

In tobacco-sensitive (tobacco idiosyncratic) individuals, intradermal injection of nicotine-free extracts of tobacco leaves or tobacco smoke gives rise to an immediate wheal and flare reaction (see Part II, below); this is an immune reaction. Stauffer[110,111] recorded the case of a female tobacco worker who suddenly developed eczema, and was found to have a high degree of skin sensitivity to tobacco, while skin tests with nicotine were negative. In subjects showing positive skin reactions to tobacco extracts, similar skin tests performed with 1:10,000 and 1:5,000 nicotine tartrate were negative (Harkavy[42a,b,46]). In one case out of 21 tested, 0.4% nicotine sulphate gave a definitely positive reaction (Sulzberger, Scholder and Feit[115]). Skin tests with dilute nicotine solution were also negative in patients with thromboangiitis obliterans (Harkavy[42a,46], Sulzberger[112]), and in patients with coronary artery disease (Harkavy[42b]), although in 312 routine patch tests on patients with dermatitis, 17 showed weak and 2 showed strong reactions to 1% nicotine salicylate (Swineford and Radford[116]). These results indicate that nicotine plays little if any role in tobacco sensitivity (Sulzberger[113b]), and Rosen[95] has expressed the opinion that skin tests with nicotine are of no value at all. Nevertheless, Harkavy[42a] did not altogether rule out nicotine as possibly playing an accessory role in sensitization to tobacco, arguing that it was well known that sensitiveness to certain drugs and chemicals might exist in spite of negative skin reactions. He reasoned that nicotine might act in one of 2 ways: (1) by combining with the body proteins to form antigenic conjugates capable of sensitizing the individual and stimulating antibodies which react specifically with the haptene, or (2) as a haptene combined with the tobacco-leaf protein; investigations of sensitivity to the latter might, therefore, imply sensitization to both nicotine and tobacco protein (Harkavy[48]). Lowell[73] drew attention to the fact that, although pharmacologically significant amounts of nicotine appeared to be absorbed in the course of smoking, this point has received little attention in assessing the role of tobacco in the production of allegedly allergic disease. It should be kept in mind that overemphasis of possible

immunological aspects of tobacco tends to minimize the importance of the pharmacological action of nicotine, and *vice versa*, and Lowell's reminder is timely. Harkavy[48] emphasized that, in evaluating the reactions of the living organism to such a complex substance as tobacco, one must take into consideration: (1) allergy to nicotine and other components in smoke; (2) sensitization to the allergenic constituents in the tobacco leaf; and (3) the pharmacologic factor in nicotine which may play a synergistic role in the allergic response of hypersensitive [that is idiosyncratic] individuals with intact sympathetic nervous systems who manifest cardiovascular abnormalities as a result of smoking.

PART II. IMMUNOLOGY OF TOBACCO AND TOBACCO SMOKE. Since tobacco leaves contain protein, it might be expected that aqueous extracts of tobacco would be antigenic and lead to antibody formation in susceptible animal species. Indeed, unequivocal demonstration of this phenomenon would seem to be essential, and basic to any study of tobacco sensitivity in man. Yet the fundamental animal experiments have not been undertaken.

Harkavy[44,45a] gave 11 male and 3 female rats intraperitoneal injections of denicotinized tobacco extracts every day for 6 to 12 weeks; 5 of the males and all 3 females received tobacco extract only, but the other males were simultaneously injected with another antigen as well (horse serum, egg white, ragweed, or meningococcus toxin). Eight of the males showed evidence of having been sensitized to tobacco, in that isolated intestinal strips from these animals contracted when tobacco extract was added to the organ (Schultz-Dale reaction); no evidence of sensitization to the other antigens was found. After the intestinal preparation was washed, addition of the same quantity of tobacco extract which had previously caused prompt contraction had no effect, indicating desensitization of the gut. None of the injected females became sensitized, and isolated intestinal strips from control animals showed no Schultz-Dale reaction to tobacco extracts. Similar results had been previously reported by Friedlander, Silbert and Laskey[32], who injected extracts of 60% denicotinized tobacco and produced gangrenous lesions of the toes in 33 of 48 male rats, but in none of 12 females. Control males living under identical conditions did not develop gangrene; nevertheless, the occasional appearance of spontaneous gangrenous degeneration in the toes of their own rats kept in cages led Thienes and Butt[118] to question the relationship claimed by Friedlander and co-workers[32] between the injected tobacco and the development of gangrene; but Harkavy[48] did not accept this criticism. Commenting on Harkavy's experimental results, Lowell[73] considered it surprising that in the male rat tobacco should be a more active antigen or allergen than either egg white or horse serum, both known to be highly antigenic in other animals, and also that there should be apparent sex differences. Harkavy's experiments were repeated by Lopes de Faria[70,71], who injected 41 white rats of various ages with nicotine-free tobacco extracts once daily for 114 days, and found the precipitin tests for tobacco in the blood serum negative at the end of the experiment. Schultz-Dale tests were made on all but 4 tobacco extract-injected rats, and on some control animals; all were negative, with the exception of one case which the author stated could be interpreted in several ways. Lopes de Faria suggested that his failure to confirm Harkavy's results might be due to the possibility that the varieties of tobacco

he employed had a much lower antigenic power than the tobaccos used by Harkavy; the former made his extracts from 4 varieties of cured tobacco leaves grown in Minas Gerais, while the latter used pooled extracts from Burley, Maryland, Virginia and Xanthis (Turkish) tobaccos. The results of Lopes de Faria appear to be the more conformable to immunological principles.

In view of the scarcity of attempts to immunize animals with preparations of tobacco, it would seem very desirable that fundamental immunological investigations of tobacco protein be made in susceptible species (for example rabbits), using as antigens both green and cured tobacco leaves, and many varieties of tobacco; and that cross-immunization studies also be made to determine whether an animal sensitized to one variety of tobacco be sensitive to another one. Similar studies should be made with tobacco-smoke extracts, since Pipes[88] has presented important evidence suggesting that allergy to tobacco smoke is an entity distinct from allergy to tobacco. Vaughan[123] suggested that different grades of pipe tobacco, cigar tobacco, and cigarettes might differ in antigenic activity; the different materials used to develop the characteristic flavor of various brands also might be antigenic. Since tobacco smoke is an aerosol, and active sensitization by means of antigenic aerosols is possible, it would be reasonable to expect to be able to induce tobacco-smoke sensitivity in animals through inhalation. An editorial in the *Medical Times* (**45**, 171, 1917) long ago pointed out that inhalation of the combustion products of tobacco afforded a very direct mode of access to the blood, and that it was reasonable to suppose that the inhalation of tobacco protein was capable of producing allergic effects. In order to equate tobacco-leaf sensitivity with tobacco-smoke sensitivity, it must be assumed that tobacco protein is not destroyed or denatured by combustion, which is highly unlikely; indeed, the work of Pipes[88] furnishes evidence for the belief that tobacco protein is altered by the processes of combustion. Rosen[95,96] further emphasized that neither tobacco-leaf nor tobacco-smoke *extracts* duplicate too well the actual smoke as it contacts the nose, mouth, and lungs. With this in mind, tobacco smoke (inhaled) should also be tried as a shocking antigen in immunological studies on animals sensitized to tobacco or tobacco smoke, or both.

Skin Reactions to Tobacco. Sulzberger, Scholder and Feit[115] observed 3 types of positive skin responses using tobacco extracts: 1) eczematous reactions; 2) immediate wheal and flare (erythema) reactions; 3) late reactions, probably of the tuberculin type. Harkavy[45] also described delayed reactions 2 to 4 days after intracutaneous injections of tobacco extract; biopsies showed an acute dermatitis. In tobacco-sensitive subjects, intracutaneous injection of nicotine-free tobacco-leaf extracts or tobacco-smoke solution may result in the development within 10 to 20 minutes of a wheal, pseudopods, and erythema. A similar reaction may occur if the injected solution is irritant or if it contains histamine. Fontana, Redisch, Nemir, Smith and Sulzberger[28] (personal communication) concluded that the tobacco extracts employed by them were not primary irritants, and that the positive wheal reactions of the skin to intradermal injections of these tobacco extracts were due to specific sensitization; and, in view of the care taken to avoid the use of extracts giving nonspecific reactions, and of the wide experience of the workers in the field, Lowell[73] was of the opinion that in most instances skin reactivity to an

extract of tobacco actually means the presence of allergy in some degree to something in the extract. (It will be recalled from the preceding discussion that this is not synonymous with saying skin reactivity to "tobacco" or to "tobacco smoke.") Using the intradermal technique, the incidence of skin sensitvity to tobacco extracts in clinically healthy adult nonsmokers has been shown to range from 12 to 21.3%; and in normal (nonallergic) children, who had never smoked nor had contact with tobacco except in the most casual way, the incidence ranged from 7.5 to 17.5% (Table 1A). Of 500 individuals tested, 79 or 15.8% showed positive skin tests to one or more of the tobacco extracts used (Fontana *et al.*[28], personal communication). The incidence of skin sensitivity in normal smokers (Table 1*B*) has been reported to be considerably higher than this by some investigators (for example Harkavy; Sulzberger), but not by others (for example Trasoff, Blumstein and Marks[121]); and the higher incidence among smokers was presumed to be a direct consequence of greater exposure to tobacco and tobacco smoke (Lowell[73]).

TABLE 1.—INCIDENCE OF SKIN SENSITIVITY TO TOBACCO EXTRACT IN NORMAL SUBJECTS

Author	*No. in Series*	*Class of Subject*	*Skin Sensitivity to Tobacco Extracts, %*
		A: Nonsmokers	
Harkavy[43a]	126	Adult females	12
Sulzberger[113b]	38	Healthy adults	16
Trasoff, Blumstein and Marks[121]	52	Normal subjects	21.3
Peshkin and Landay[84]	200	Normal children	17.5
	150	Normal children	16.7
Lima and Rocha[69]	200	Normal male children	7.5
		B: Smokers	
Harkavy, Hebald and Silbert[51]	107	Cigarette smokers	9.3
Harkavy[42a]	166	Male cigarette smokers	8.4
Harkavy[43b]	200	Normal smokers	38*
Harkavy and Romanoff[49]	100	Male smokers	11†
	100	Female smokers	
Harkavy[46]	400	Normal smokers	9†
Sulzberger[112]	38	Healthy male and female smokers	36
Sulzberger[113b]	50	Healthy adults	36
Trasoff, Blumstein and Marks[121]	40	Normal smokers	17.5
		C: "Normal Controls," Smoking Habits Not Specified	
Romanoff and Rubin[94]	68	"Normal controls"	16†
Green[37]	100	"Healthy controls"	13†

* Also sensitive to other allergens (see text).

† Sensitive to tobacco alone (see text).

Harkavy[48] reviewed studies on skin sensitivity to tobacco extracts in clin- others, and stated: "These findings indicate that about 3 times as many smokers react to tobacco extracts as compared with nonsmokers. This is in accord with the well-known fact that repeated exposure to a given allergen may result in the development of positive skin reactions as well as circulating antibodies indicative of an immune

response. . . . In tobacco we have an allergen which permeates the atmosphere 365 days a year, a period which exceeds the pollenating season in many communities. Hence, the sources of exposure and possible sensitization are to be found not only in smoking but also in tobacco dust in the atmosphere."

In smokers from among hospital populations, the incidence of positive skin tests to tobacco extracts was of the same order as that reported by the same authors for normal smokers (Table 1C). Bowman[10] found an even higher incidence of positive tobacco reactions in a group of 23 normal nurses (whose smoking habits were not, however, given) than in a group of 60 normal male smokers. It is interesting to note that Lima and Rocha[69] found the incidence of positive skin reactions to tobacco to be 30% among the children whose mothers smoked, as compared to 13.4% where only the fathers smoked; this suggested to these authors that sensitization *in utero* or by way of the maternal milk might have occurred.

Since skin sensitivity to tobacco extracts is often accepted as a measure of tobacco allergy, it is necessary to consider the subject in the light of multiple sensitivity or the allergic constitution. Trasoff, Blumstein and Marks[121] found 25% of 24 allergic nonsmokers giving positive skin tests to tobacco extract, compared to 47.1% of 17 allergic smokers in whom tobacco played no part in their clinical manifestations. In a series of 53 asthmatic children aged 3 to 12 years, 88.7% gave positive skin reactions (Chobot[18]), and in another series of 164 allergic children aged 2 to 15, 69.5% showed positive reactions (Peshkin and Landay[84a,b]), the latter figure being about four times as high as in the control series of nonallergic children. Lima and Rocha[69] found 17.5% of 200 allergic children aged 2 to 13 showing positive skin tests to tobacco extract, compared to 7.5% of an equal number of nonallergic male children aged 5 to 13.

Harkavy and Romanoff[49] tested the skin reactions of 100 normal male smokers and 100 normal female smokers to tobacco, ragweed, and timothy pollen; 11% were sensitive to tobacco alone, and 27% to the three allergens in various combinations. Twenty-seven of 76 tobacco-sensitive subjects also gave various skin reactions to foods and inhalants (Harkavy[42]), and Harkavy[43b,46] considered that such reactions in normal persons might be an expression of a general allergic response which is usually characterized by a multiple sensitization. Multiple sensitization was also found in patients with thromboangiitis obliterans (Harkavy, Hebald and Silbert[51]). According to Trasoff, Blumstein and Marks[121], 11 of 52 normal nonsmokers reacted slightly to tobacco, 9 to house dust and feathers, and 2 to timothy extract; less than 50% of patients with thromboangiitis obliterans or allergy reacted to tobacco extracts, while almost all reacted to one or more of the other allergens tested (ragweed, timothy, house dust, feathers). In Peshkin and Landay's[84a,b] series of 200 nonallergic children, 35 or 17.5% showed skin sensitivity to tobacco, and of these 35, 83% also showed skin sensitivity to pollen. Of 150 nonallergic children, 25 or 16.7% showed positive skin tests to tobacco extract, and 23 or 92% of these latter also showed skin sensitivity to dust. No correlation could be found in these nonallergic children between sensitivity to tobacco antigen and sensitivity to dust antigen, but in allergic children similarly tested with tobacco and pollen, a parallelism was found between the two, the positive reaction to tobacco being more closely bound up with the positive reaction to pollen

than *vice versa* (Peshkin and Landay[84a,b]). Green[37] reported that 13% of 100 normal controls (smoking habits not given) reacted to tobacco alone, 15% to pollen-dander alone, and 32% to the combination; 40% were nonreactors. The relatively low incidence of skin sensitivity to tobacco extracts alone (11% and 9%, respectively) should be contrasted with the 38% incidence of positive reactions in normal smokers (Harkavy and co-workers; see Table 1*B*), the difference, as Harkavy himself pointed out, being due to multiple sensitivity; it is thus a moot question whether the figure of *ca.* 10% or of *ca.* 40% represents the truer measure of tobacco sensitivity in the absence of data concerning the presence of reagins to tobacco in the positive reactors.

Antibodies in the serum of individuals showing skin sensitivity may be identified by passive transfer of such serum into the skin of nonsensitive individuals, the so-called Prausnitz-Küstner reaction, in which blood serum from an allergic individual is injected into the skin of a normal individual; 6 hours to several days later, the prepared site shows an urticarial reaction when injected with the antigen to which the donor is allergic, while other parts of the recipient's skin show no response. (The Prausnitz-Küstner reaction is also referred to as the "passive transfer" test, the antibody being transferred from the reactor to the normal recipient also being termed a "reagin.") A substantial proportion of individuals with skin sensitivity to a given antigen have antibodies in their serum capable of giving a Prausnitz-Küstner reaction, and this reaction is highly specific for the antigen (allergen) in question.

Using the passive transfer technique, Harkavy and his co-workers claimed to have frequently demonstrated the presence of tobacco reagins in patients giving positive skin tests to tobacco extracts (Harkavy, Hebald and Silbert[51]; Harkavy[42a,b]; Harkavy and Witebsky[50]; Harkavy[45b,46]). On the other hand, Sulzberger and his colleagues could not regularly demonstrate the presence of reagins in the serum of reactors (Sulzberger, Scholder and Feit[115]; Sulzberger and Feit[114]; Sulzberger[113b]), and he expressed himself as being in complete disagreement with Harkavy (Sulzberger[113]). Thus, Sulzberger considered allergy to tobacco to be nonatopic, while Harkavy considered it atopic. In general, other workers have confirmed the findings of Sulzberger rather than of Harkavy. Ehrenfeld and Sturtevant[24,25] reported negative Prausnitz-Küstner reactions in tobacco-sensitive peptic ulcer patients; Trasoff, Blumstein and Marks[121] could demonstrate no reagins in the serum of tobacco-sensitive patients with thromboangiitis obliterans or coronary disease, nor in normal nonsmokers who reacted to tobacco, but did obtain one positive transfer in 6 allergic nonsmokers who showed slight reactions to tobacco; Westcott and Wright[129] obtained a positive result in only 1 of 8 passive transfer experiments using the sera of tobacco-sensitive thromboangiitis patients; Bowman[10], studying thromboangiitis patients who were sensitive to tobacco as well as to other allergens (ragweed pollen, timothy pollen, horse dander), found that, with one exception, passive transfer of the tobacco reagins was successful only when a positive passive transfer was obtained with one or more of the allergens tested. In several studies on allergic children, Chobot[18] selected the 4 most marked reactors to tobacco for testing, and reported that passive transfer tests were negative; in contrast, Peshkin and Landay[84a,b] found that 72% of the allergic children in their

series with positive skin reactions to tobacco also gave positive Prausnitz-Küstner reactions, while children with negative skin reactions had negative passive transfer tests. Lima and Rocha[69] tested skin reactions to tobacco in 200 nonallergic and 200 allergic children; 15 of the former and 35 of the latter showed positive skin reactions, and in 6 and 15, respectively, reagins could be demonstrated in the blood; tests for reagins in children with ± skin reactions were negative. The specificity of the reagins to tobacco allergy and the nonexistence of cross-reactions between this allergen and the allergens of pollens (grass and ragweed) could be clearly demonstrated by passive transfer studies: none of the other allergens to which children were already sensitized were capable of exhausting or neutralizing the tobacco reagins found in the 15 allergic and 6 nonallergic children.

Lowell[73] has pointed out that the lack of accord among the workers in the field regarding the presence or absence of tobacco reagin in tobacco-sensitive subjects is important because there is no known mechanism other than an antigen-antibody reaction which can explain this phenomenon. However, he added, even a single instance in which the phenomenon was unequivocally demonstrated would suffice to establish allergy to tobacco as an entity. Harkavy[48] listed several postulates necessary to demonstrate the allergenicity of tobacco: (1) the test substance must be nonirritative in nature and, therefore, give negative reactions in nonallergic persons who have had no previous contact with it; (2) positive skin reactions should be accompanied by specific reagins (antibodies), at least, in some of the reacting individuals; (3) the whealing response characteristic of the immediate reaction should manifest local esosinophilia on histologic section; and (4) it should be possible to elicit specific clinical symptoms in the patient after exposure to the reacting substance, followed by their disappearance upon its withdrawal. After a review of the literature on the subject, Harkavy[48] decided that all of the above postulates had been satisfied. It is, indeed, beyond question that allergy to tobacco extracts, presumably atopic in nature, is an established fact, although the true incidence of such allergy is far from clear. Westcott and Wright[129] have maintained that the high incidence of positive skin reactions to tobacco extract reported by other investigators, and interpreted by them as allergic reactions, was due to some of the many nonspecific skin reactions from chemical irritation; and Sulzberger[112] believed that both skin tests and reagin demonstrations with an allergen were of significance only in conjunction with and in support of clinical evidence pointing to the same substance. Obviously, immunological signs of tobacco allergy may exist without symptoms, and symptoms of tobacco allergy may be present in the absence of demonstrable immunological signs.

Further evidence that skin sensitivity to tobacco extracts may be a true immunological reaction is furnished by the demonstration of specific absorption of tobacco reagins by corresponding tobacco allergens in cases with multiple sensitization. Skin tests performed by Harkavy and Witebsky[50] on a subject who had used snuff for 35 years gave 4+ reaction to tobacco and 1+ to ragweed, and passive transfers made with whole serum showed marked reagins for tobacco and none for ragweed. Serum was then mixed with tobacco extract in various dilutions, the mixtures injected into the skin of normal receptors, and the site tested 24 hours later with tobacco ex-

tract, when negative reactions were obtained, indicating absorption of tobacco antibody by the tobacco antigen. A second patient gave multiple skin reactions in different degree for timothy, tobacco, and corn. Preliminary addition of timothy extract to serum *in vitro* exhausted the reagin for timothy and corn, but not for tobacco; previous addition of tobacco extract to the serum fixed the reagin for tobacco and corn, but not for timothy; addition of corn extract to this serum absorbed the corn antibody, but did not influence the tobacco or the timothy antibody, indicating that the reagins for timothy and tobacco were specific and independent, and consequently that the skin reactions were due to an antigen-antibody mechanism. In this connection, experiments reported by Pipes[88] are significant. Passive transfer of serum from an individual giving a positive history of allergy to tobacco as well as a 4+ positive skin reaction to tobacco extract was made by this worker into three nonallergic subjects; these sites could be exhausted by tobacco extract, but were only 30 to 40% exhausted by tobacco-smoke extract. Similar passive transfer experiments were made with serum from an individual giving a positive history of allergy to tobacco smoke and a 4+ skin reaction to tobacco-smoke extract; these sites could be exhausted with tobacco-smoke extract, but only 40 to 50% exhausted by tobacco extract.

Harkavy and Witebsky[50] found that the tobacco antigen diluted 1:10,000 gave positive reactions when tested in a site passively sensitized with whole serum, and that undiluted antigen solution elicited positive reactions with serum dilutions through 1:125; such a degree of serum as well as of antigen dilution was considered by them suggestive of the specific nature of the reaction.

Harkavy[45b] described the induction of a characteristic local eosinophilic reaction in the immediate urticarial response in patients giving positive skin tests with tobacco extracts; biopsy studies of the immediate and late skin reactions to tobacco and saline controls showed eosinophils, varying in number, in the histologic sections of the immediate urticarial wheals produced by the tobacco allergens in 7 of 9 cases. This eosinophilic response was considered by Harkavy as further evidence of the specificity of the skin reactions to tobacco extracts, but Lowell[73] stated this demonstration begs the question so far as any causal relationship between skin tests and disease is concerned. In connection with the eosinophilic response reported by Harkavy, it may be mentioned that Deschiens and Poirier[21] found that aqueous extracts of dried tobacco leaf given hypodermically every other day for at least 18 days were eosinophilogenic in the guinea pig after a latent period of 5 to 6 days; this reaction may have represented an immunological response, and may offer a hint to the direction of future work.

In tobacco workers giving histories of dermatitis, patch tests using tobacco leaves were largely negative (Rutledge[100]; Franchi[29]; Schwartz[104]; Vero and Genovese[124]; Samitz, Mori and Long[101]); patch tests made with paste used in the manufacture of cigars were positive (Franchi[29]), and a mixture of paste and tobacco leaf gave stronger skin reactions than leaf alone (Vero and Genovese[124]).

Evidence that the allergen in tobacco giving rise to skin sensitivity is not nicotine has been presented in Part I. It may be added in this connection that Wright and Moffat[131] found no direct relationship between the degree of fall in peripheral surface temperature following smoking, and skin tests

for tobacco and nicotine. Maddock, Malcolm and Coller[75] found no consistent difference in the skin temperature following smoking between 10 individuals who showed skin sensitivity to tobacco extracts, and 19 individuals who showed no such sensitivity; these authors considered that the 2 effects (namely, peripheral vasoconstriction and skin sensitization) were due to different components of tobacco; and it has been amply demonstrated that the peripheral temperature drop following smoking may be duplicated by appropriate nicotine injection.

According to Sulzberger[113b], that part of the tobacco which elicits the skin reaction of idiosyncracy is coctostabile and thermostabile, and is not destroyed by ultraviolet rays or Roentgen rays in the dosages employed.

Harkavy and his colleagues used aqueous extracts prepared from Burley, Maryland, Virginia, and Xanthis (Turkish) tobaccos; subjects who were reported as hyper-reactors to tobacco showed positive skin reactions to one or another of these 4 extracts. Sulzberger[112] employed 10 different extracts of tobacco, and found that any given patient showing a positive skin test to tobacco might not react to all the tobacco extracts, but might be sensitive to a given mixture and not sensitive to any other mixture; he suggested that it might be possible to discover which particular tobaccos gave the least number of positive reactions, and thus determine which tobacco was least likely to prove injurious. He instanced a case in which patch tests were positive with tobaccos from one brand of cigarette, while patch tests with other brands were negative. Six of the extracts were prepared by the usual methods of extraction (Coca) from standard brands of cigarettes and pipe tobaccos; the extracts were all denicotinized. Fontana and co-workers[28], in a personal communication stated that in a group of 79 individuals giving positive skin tests to tobacco extracts, 40 showed positive reactions to extracts made of Burley tobacco, 37 to Turkish, 45 to Virginia, 47 to mixed extracts, and 30 to *defatted* mixed extracts. Since there were more reactions to the mixed brands of tobacco extract which was not defatted, and fewer reactions to the defatted mixed extract, the defatting process was considered to have removed an appreciable quantity of esters, aldehydes, and other organic compounds ordinarily thought to be volatilized in the smoke of the cigarette. The findings suggested that a large number of individuals reacted to this volatile aromatic fraction alone. In the group of positive cases tested, the individuals reacted to from one to 5 of the extracts; this would indicate that a person yielding positive skin tests might prove sensitive to only one specific type of tobacco, and not to any other type. Trasoff, Blumstein and Marks[121] used extracts made according to the method of Coca of various brands of common cigarettes and cigars; Green[37] employed extracts of Burley, Virginia, Maryland, Xanthis and mosaic tobaccos prepared according to the same method; Allen, Barker and Hines[2] used extracts from tobacco in the commonly-smoked brands of cigarettes; Lima and Rocha[69] employed extracts prepared from Burley, Maryland, Xanthis, Virginia, Havana, and Baiano tobaccos; Rosen[96] used extracts from assorted domestic and Turkish leaf tobacco. In an attempt to determine whether the skin reaction to tobacco extract represented a specific, qualitative, atopic hypersensitivity [that is, an idiosyncrasy], or was merely a manifestation of a less discriminating capillary phenomenon as produced, for example, by histamine, Lounsbury and Oughterson[72] pre-

pared 4 fractions by chemical procedures from the green leaf of Connecticut shade-grown tobacco: (a) adenine sulphate fraction; (b) histidine fraction; (c) arginine fraction; (d) lysine fraction; and they compared these 4 fractions with (e) an extract of whole green tobacco leaf, (f) an extract of cured tobacco, and (g) histamine in 1:5000 and 1:500,000 dilution. In intradermal tests with (a)–(g) made on 100 selected subjects, no direct relationship was found to exist between any 2 of the excitants used. The lack of correlation between the green-leaf extract and the cured leaf was thought to point toward the possibility that there was some component of cured-leaf extract, not present in the green-leaf extract, which might be responsible for the specificity of the tobacco reaction in tobacco-sensitive subjects with reagins in the blood. The authors suggested, although they themselves did not do this, that this point should be checked by using an extract of the green leaves of the same tobaccos used in the preparation of the cured-leaf extract.

Harkavy[43b] investigated the incidence of skin sensitivity to tobacco leaf, tobacco pollen, and tobacco seed in 200 normal smokers and in 69 cases of thromboangiitis obliterans. Of the 200 controls, 30% reacted to the tobacco pollen and seeds, while 38% reacted to the leaf; in the thromboangiitis group, 59% reacted to the pollen and seed, and 78% to the tobacco leaf. In the 200 controls, no reactions to tobacco pollen or seeds occurred without a concomitant reaction to ragweed or timothy pollen extracts; this was interpreted as indicating that reactions to tobacco pollen as well as tobacco leaf in normal smokers may be an expression of a general allergic response. Ratner[91] (quoted in Peshkin and Landay[84a]) advanced the possibility that positive skin reactions using tobacco extracts might be due to contaminating pollens deposited on the tobacco leaf during its cultivation and growth in the field. This possibility may be minimized, however, for careful examination of several brands of cigarettes by Kaufman[62] (unpublished observations) failed to reveal any pollen grains.

Positive skin reactions to intracutaneous injection of aqueous tobacco extracts presuppose that tobacco allergens are soluble, but other possibilities should be kept in mind. For example, Swineford and Radford[116] found, in 312 routine patch tests on patients with dermatitis, that 28 showed weak and 5 strong reactions to tobacco (cigarette and pipe) oil. And there is the old observation reported by Chevallier[16] of a man who smelled with pleasure a large amount of good Spanish tobacco, then lost consciousness and became covered with a cold sweat. Many such (presumably allergic) reactions to various odors have been recorded as curiosities by Gould and Pyle[35], but that should not preclude their immunological investigation. According to Chevallier[17], new tobacco workers may at first be disagreeably affected by the odor *sui generis* which exists in tobacco factories; and Samitz, Mori and Long[101] stated that roughly one in every thousand cigar workers was found to be susceptible to the smell of tobacco. Recently, Schirren[102] reported a case of allergy in the form of an acute dermatitis of the face incited by a tobacco perfume.

Mention may be appropriately made here of tobacco-associated, but non-tobacco allergens. In Schirren's case cited above, the patient was also sensitized to tobacco and wood smoke and to coffee aroma. Believing that the common factor might be the presence of diacetyl in all of these materials, Schirren performed a patch test with

this substance, and obtained a strongly positive reaction. Newman[82] reported the case of a patient, an inveterate cigarette smoker with dermatitis of the left upper lip and left index and middle fingers, who proved to be sensitive to tobacco from cigarettes containing diethylene glycol, and also to a 5% solution of diethylene glycol itself. Rappaport and Hoffman[90] cited the contrary case of another heavy cigarette smoker with hives first appearing on the lips and fingertips of his right hand; when this patient changed to diethylene glycol-treated cigarettes, the hives did not appear; but when a small amount of glycerol was introduced into these cigarettes, hives recurred: presumably, the patient's former brand had contained glycerol, to which he was sensitive. Vaughan[123] instanced a patient, allergic to a number of antigens, who had chronic indigestion as long as he smoked cigars known to have corn syrup in the wrapper; but when he changed to more "high grade" cigars, his indigestion disappeared. Also pertinent here are the studies of Barksdale[4,5a,b,c] on exfoliative dermatitis due to arsenic, the source of which was thought to be tobacco. Sulzberger, however, in his discussion of Barksdale's[5a] paper, stated that in his own experience hypersensitivity [that is, idiosyncrasy] to arsenic occurred only when the arsenic was in organic combination.

In regard to factors influencing skin sensitivity to tobacco, Lima and Rocha[69] stated that age and sex did not appear to be of importance in the incidence of positive skin reactions to tobacco in nonallergic children aged 5 to 13 years. Sulzberger[112] found the distribution of positive tests about equal between the sexes in 38 healthy male and female smokers. Harkavy[42b] reported that the average age of 13 patients with coronary artery disease who reacted to tobacco was 45, while the average age of 23 patients with such disease who did not react to tobacco was 60. The relationship of skin sensitivity to tobacco and certain diseases will be discussed below.

The demonstration by Pipes[88] that allergy to tobacco smoke is not equivalent to allergy to tobacco leaf has already been referred to. This author reported that 13% of 370 consecutive allergic patients (smoking history not noted) gave positive skin tests to tobacco-smoke extract, while the same extract gave negative tests in 10 nonallergic controls. Despite the small number of controls, Pipes[88] nevertheless suggested that allergy to tobacco smoke could not be adequately controlled by hyposensitization with tobacco extract alone. Harkavy[42a,b] found that some patients with coronary artery disease or thromboangiitis obliterans had positive skin reactions to tobacco-smoke extracts, but Bergman and Rikles[6] reported only negative skin reaction to tobacco-smoke solutions in their 4 cases of thromboangiitis obliterans. Clearly, the amount of investigative work done with tobacco-smoke extracts is far from sufficient. With the caution of Rosen[95,96] in mind that neither tobacco-leaf nor tobacco-smoke extract duplicates too well the actual smoke as it contacts the nose, mouth, and lungs, it would appear that, with the exception of investigations into contact dermatitis, extracts of tobacco smoke might be relatively more nearly representative of the conditions of actual smoking than would extracts of tobacco leaf.

Skin Sensitivity to Tobacco in Disease. In the absence of any demonstration of specific reagins in the serum of patients showing positive skin reactions to tobacco or smoke extracts, the value and significance of such skin reactions depend largely upon the results of

similar tests performed in statistically adequate numbers upon properly selected control subjects. Three types of normal control series have been employed: (I) known nonsmokers, (II) known smokers, and (III) subjects whose smoking history was either not known or not recorded. In addition to the foregoing, comparisons have been made with (IV) "control" series of patients with various diseases other than the one under investigation. It should be obvious that so-called controls in (III) and (IV) furnish in fact no valid control data at all, since a precise statement of the smoking habits of every subject or patient is essential in any investigation of the relationship between tobacco and any pathological or physiological change; moreover, diseased individuals may not be considered "normal controls" even in relation to patients with a presumably entirely different form of disease: all disease alters the sufferer in *nonspecific* as well as in specific ways. These factors should be kept in mind during the following discussion.

Harkavy, Hebald and Silbert[51] first reported that patients with *thromboangiitis obliterans* (TAO) showed a much higher incidence of positive skin reactions to tobacco extracts than did normal smokers or nonsmokers, and the presence of atopic reagins to tobacco was demonstrated in 13 of 20 patients by means of passive transfer. The number of TAO patients investigated was gradually extended, and essentially similar results obtained

TABLE 2.—INCIDENCE OF SKIN SENSITIVITY TO TOBACCO EXTRACTS IN PATIENTS WITH THROMBOANGIITIS OBLITERANS

Author	*No. in Series*	*Class of Subject*	*Skin Sensitivity to Tobacco Extract, %*
A: "CONTROL" PATIENTS, WITHOUT TAO			
Harkavy[43a]	319	Male smokers, excluding TAO	20
Sulzberger[112]	153	Hospital population, excluding TAO	28
Sulzberger[113b]	95	Hospital population, excluding TAO	36
Westcott and Wright[129]	35	Patients suffering from a variety of ailments, excluding TAO	48
Allen, Barker and Hines[2]	?	"Control cases"	33
B: PATIENTS WITH TAO			
Harkavy, Hebald and Silbert[51]	68		85.4
Harkavy[42a]	87		87.3
Harkavy[43a]	103		86
Harkavy[46]	140		78
Sulzberger, Scholder and Feit[115]	9		77.8
Sulzberger and Feit[114]	22		86.4
Sulzberger[112]	13		77
Sulzberger[113b]	24	Not atopic	78
Bergman and Rikles[6]	4		0
Trasoff, Blumstein and Marks[121]	31	Proven cases	16.0
Westcott and Wright[129]	35		42.8
Romanoff and Rubin[94]	52		50
Högler[55]	?		83
Wood[130]	?		(not increased)
Green[37]	44	Proven cases	66
Allen, Barker and Hines[2]	?		35
Bowman[10]	69		(insignificant difference from controls)

(Harkavy[42a,43a,46]). Sulzberger and his colleagues confirmed the findings of Harkavy that there was a markedly higher percentage of cutaneous sensitivity to tobacco in TAO than in any other group, but failed to demonstrate reagins in the serum of hyper-reactors with any regularity (Sulzberger, Scholder and Feit[115]; Sulzberger and Feit[114]; Sulzberger[112,113a,b]). A much higher than normal incidence of positive skin reactions in TAO was also noted by Romanoff and Rubin[94] (cited by Harkavy[46]) and by Green[37]; according to these investigators, the percentage of reactors to tobacco in patients with this disease varied from about 50 to 66, compared to a normal incidence of under 40% in non-TAO populations. Högler[55] stated that 83% of patients with TAO showed positive skin reactions to tobacco. Other authors, however, reported their failure to demonstrate a higher incidence of specific cutaneous sensitivity to tobacco in TAO patients than in "controls" (Bergman and Rikles[6]; Trasoff, Blumstein and Marks[121]; Westcott and Wright[129]; Wood[130], cited by Thies and Freeland[130]; Allen, Barker and Hines[2]; Bowman[10]) (Table 2*B*, compared to Table 2*A* and Table 1). It is difficult to reconcile these conflicting reports, although attempts to do so have been made on, so to speak, both sides. For example, Harkavy[46] dismissed the results of Trasoff, Blumstein and Marks[121] on the grounds that these authors had failed to show proof that the cases studied were "proved cases of thromboangiitis obliterans," and he criticized the results of Westcott and Wright[129] on the grounds that the types of cases the latter authors chose as controls consisted of patients who presented vascular syndromes previously reported by Harkavy as additional forms of clinical manifestations of tobacco hypersensitiveness [that is, an idiosyncrasy] other than TAO. Later, Harkavy[48] reinterpreted the data of Westcott and Wright, and maintained that these were, after all, in agreement with his own conclusions. Westcott & Wright[129], on the other hand, believed that many of the so-called positive skin reactions to tobacco were nonspecific reactions from chemical irritation. Walzer[126] questioned the validity of a comparative study of skin tests in which one of the groups consisted of patients with a vascular disturbance (that is, TAO patients), since the state of the circulation is a vital factor in wheal formation, and hence any variation from the normal which may be detected in skin tests in TAO may rest on circulatory factors and not on immunological ones; he also doubted the specificity of many of the reportedly positive reactions. It would appear that the objections raised on either side are all valid to a greater or lesser degree.

Three possible reasons have been suggested for the high incidence of positive skin reactions to tobacco in thromboangiitis obliterans: (1) that tobacco allergy plays an exciting, or even etiological role in this disease; (2) that multiple sensitivity exists in TAO patients, idiosyncrasy to tobacco being but one of several or many allergies present; and (3) that, since patients with TAO tend to be heavy smokers and hence more intensely exposed to tobacco and tobacco smoke than "normal" individuals, a high incidence of skin reactivity to tobacco extracts is only to be expected. The first view has been championed mainly by Harkavy, who, in his earlier papers, appears to have inclined to the belief that tobacco plays an etiological role in TAO, but in a later report (1939) he summed up his position as follows: "Whether tobacco allergy is the only factor operative in the precipitation of thromboangiitis obliterans and certain

types of coronary artery involvement; or whether it is an important synergist in an underlying metabolic disturbance, or perhaps some nonspecific infection which may have acted as a preparatory agent in establishing these vascular shock organs similar to that which obtains in the Schwartzmann phenomenon, it is impossible to say. [The] exciting role [of tobacco] however, is suggested immunologically and clinically by the evidence presented." Recently, Harkavy[48] reviewed the speculations of a number of other authors as to the etiology of this condition, and added: "In view of the consensus of opinion as to the importance of tobacco in this disease and the fact that only a small percentage of excessive smokers develop thromboangiitis obliterans compared with the number of people who smoke as much, it is logical to assume that hypersensitiveness [that is, idiosyncrasy] to tobacco, on the one hand and the presence of a specific vascular shock tissue, on the other, may constitute the basic elements in the pathogenesis of this disease." Comment on Harkavy's earlier work was extensive. Brooks[11] failed to see anything in the results of Harkavy[42] showing any definite proof insofar as etiology of this disease was concerned. Conner[20] found it very hard to understand why, with excessive tobacco smoking so common, TAO should be relatively so rare; it seemed evident to him that at best hypersensitiveness [that is, idiosyncrasy] to tobacco could be only one of a group of factors which must include at least those of race, age, and sex. Trasoff, Blumstein and Marks[121] raised the related point that, if hypersensitivity [that is, idiosyncrasy] played a role in TAO and coronary disease, why there should be so few cases of these diseases among allergic individuals; in their experience, the proportion was no larger than among nonallergic individuals. In a critical review of Harkavy's earlier work, Walzer[126] concluded that, in view of the fact that so many features of the tobacco hypersensitivity [that is, idiosyncrasy] theory of TAO were contrary to immunological and clinical precedent, one could not be blamed for manifesting a reluctance to accept this hypothesis until stronger evidence was produced in its favor. Those investigators who found no higher incidences of skin sensitivity to tobacco in TAO patients than in control subjects obviously did not subscribe to Harkavy's theory (for example, Bergman and Rikles[6]; Westcott and Wright[129]; Wood[130]; Allen, Barker and Hines[2]; Coca[19]). Sulzberger at first believed that his results lent strong support to the theory (Sulzberger, Scholder and Feit[115]), but later assumed that, since other allergens also elicited positive skin responses, cases of TAO must exist in which other excitants and not tobacco were the major factors (Sulzberger[113b]). Summing up, Lowell[73] declared that the available evidence for allergy to tobacco as a cause of thromboangiitis obliterans and other forms of vascular disease was so inconclusive that [in 1955] the question remained a matter for speculation only. It should, however, be pointed out here that, regardless of views held concerning etiology, there is almost unanimous agreement among clinicians that smoking does aggravate thromboangiitis obliterans; and virtually all reported series of cases show a preponderance of heavy smokers.

Multiple sensitization in patients with thromboangiitis obliterans has been reported by several investigators (Harkavy, Hebald and Silbert[51]; Harkavy[42]; Harkavy and Romanoff[49]; Harkavy[46]; Sulzberger[113]; Green[37]; Trasoff, Blumstein and Marks[121]; Bowman[10]). Tobacco was said by

Harkavy, Sulzberger, and Green to be the principal allergen causing positive skin reactions in TAO, although Bowman reported that the incidence of positive reactions to ragweed pollen, timothy pollen, and horse dander, as well as to tobacco extract, was approximately equal in 69 TAO patients and in 60 normal male smokers, indicating the same distribution of allergy in the 2 series. Romanoff and Rubin[94] (cited by Harkavy) found multiple sensitivity to tobacco and other allergens in normal controls and in TAO patients, but more marked in the latter group. The skin response to 1:1000 histamine was slightly greater in a group of patients with TAO than in normal male smokers; there was a slight excess in the number of 1+ reactions to tobacco in the TAO group over the control group, and it was suggested that this difference might possibly have been due to the same factor which was responsible for the increase in the response by the TAO group to the nonspecific excitant, 1:1000 histamine (Coca[19]). Lowell[73] deemed it unnecessary to postulate a more intense or a different type of allergic reaction in those skin-sensitive patients with TAO, as compared with those who did not have the disease but also reacted to tobacco by skin test.

Lowell[73] also raised a question fundamental in defining the relationship of tobacco or smoking to any disease, in these words: "If an allergic reaction to tobacco is the cause of [thromboangiitis obliterans] in those who are skin-sensitive, what cause is postulated for the 20-odd % of patients with thromboangiitis obliterans who are not skin-sensitive to tobacco?" In reply to this question, Harkavy[48] stated that "the answer of course is tobacco. This," he amplified, "was indicated by the fact that the disease in the negatively reacting patients was arrested when they stopped smoking and recurred on its resumption. Apparently, Lowell forgets that negative skin reactions in allergic patients who are clinically sensitive to various substances are a common occurrence. . . . It is well-known that in instances of allergy to drugs, it is especially difficult, and often impossible, to obtain any positive skin reactions, even though all the clinical evidence points to hypersensitiveness [that is, idiosyncrasy] to the chemical in question. Yet, one cannot attribute allergy to drugs simply to chemical irritation." According to Fontana *et al.*[28] (personal communication), vascular symptoms were reported twice as often in volunteers with positive skin tests to tobacco as in those with negative skin tests (22% of only 29 individuals being compared to 11% of 421 individuals, respectively). Smokers with positive skin tests had 2.5 times more vascular symptoms than smokers with negative skin tests. In nonsmokers, the incidence of vascular symptoms was about the same whether the skin test was positive or negative. There was said to be a significant correspondence between positive skin tests to tobacco and clinical peripheral vascular symptomatology. "It would seem, therefore, that these results are highly suggestive that a possible immunologic phenomenon is responsible for the sensitization of the vascular tree to tobacco and for certain of its abnormal or excessive responses to tobacco smoking. These findings would tend to confirm Sulzberger and Harkavy and coworkers in their concept that certain tobacco effects should be regarded as allergic sensitizations rather than as poisonings."

Lowell found especially surprising the absence of attempts to exclude nicotine as a cause of symptoms among those with vascular diseases in cases where the relationship between smok-

ing and progression of the disease (along with skin sensitivity to tobacco extracts) was offered as evidence for an allergic cause of the condition. In this connection, Trasoff, Blumstein and Marks[121] who frequently noticed a direct relationship between vascular crises and smoking, whether symptoms occurred in the lower extremities or in the coronary system, concluded that the explanation of symptoms must rest on a pharmacological basis only, that is, on the presence of nicotine rather than on immunological sensitization to tobacco protein.

Harkavy[46] considered that immunological and clinical evidence suggested that tobacco played an exciting role in coronary artery disease; of 100 patients with coronary artery disease and angina pectoris, 44 showed positive skin reactions to tobacco, and out of a group of 14 cases, 71% had reagins to tobacco. Two cases were tested with extracts of tobacco smoke, and both gave positive reactions (Harkavy[42b]). This author found it impossible to say whether tobacco allergy was the only factor operative in the precipitation of certain types of coronary involvement, or whether it played some synergistic role. Green[37] concluded that tobacco had an etiologic role in the production of the anginal syndrome in certain susceptible individuals whose shock organ was the coronary vessels; of 18 patients with coronary artery disease, 13 reacted to tobacco extracts alone, 2 to combinations, and 3 were nonreactors. Trasoff, Blumstein and Marks[121] found only 3 reactors in 23 cases of proved coronary artery disease, and were unable to demonstrate the presence of reagins in the serum of these.

So far as other cardiovascular conditions are concerned, in a series of 15 patients who suffered from circulatory disturbances in their lower extremities of an arteriosclerotic nature, and who were all excessive cigarette smokers, Harkavy, Hebald and Silbert[51] found skin tests to tobacco negative. Green[37] reported that of 31 patients with peripheral vascular disease due to arteriosclerosis (smoking habits not given), 16 reacted to tobacco extracts alone, 1 to pollen only, 3 to the combination, and 11 were nonreactors. Sulzberger[112] found 17% positive skin reactions to tobacco in 35 hypertensive and atherosclerotic heart disease cases; and Harkavy[42a], in 26 patients with circulatory disturbances of the extremities of an atherosclerotic basis, only 1 reacted to tobacco. Studying 5 patients with migrating phlebitis in whom infections were ruled out, Harkavy[42a] reported that skin tests in 1 were completely negative, 2 reacted to tobacco, while 4 showed multiple sensitizations to various foods and inhalants. In a case of migrating phlebitis which gave immediate urticarial reactions to tobacco extracts, biopsy showed the presence of eosinophils in the immediate urticarial wheal (Harkavy[45b]), a cellular reaction characteristic of an allergic response; but according to Lowell[73], this does not establish a causal relationship between the skin tests done with tobacco extracts and the exacerbations of the phlebitis. In rebuttal, Harkavy[48] pointed out that this patient showed an immediate positive skin test to tobacco extract and also a delayed reaction, eczematous in nature. The patient discontinued smoking, and the phlebitis subsided; 2 weeks after the phlebitis had completely disappeared, the patient was retested, and the phlebitic nodules developed 2 days afterwards. Harkavy considered that the exacerbation of symptoms was a result of the injection with tobacco.

Harkavy[47] had discussed clinical manifestations of polyvalent sensitiza-

tion (including sensitization to tobacco) in vascular allergy, and this author has more recently (1957) reviewed the literature on tobacco allergy in vascular disease. In his view, the literature indicates that smoking was associated in a certain percentage of atopic and nonatopic individuals with clinical signs and symptoms referable to the cardiovascular system. These may be in the form of migrating phlebitis, intermittent claudication, and thromboangiitis obliterans in the peripheral, cerebral, and coronary arteries. In cases where the heart is the major shock organ, the symptoms following the use of tobacco may be characterized by various arrhythmias, tobacco angina, and angina pectoris with coronary insufficiency, and coronary disease with or without thrombosis.

In *allergic states*, the incidence of positive skin reactions to tobacco was reported to be 88.7% in asthmatic children aged 3 to 12 years who were allergic in some way, either to proteins or to the products of bacteria (Chobot[18]); 69.5% in allergic children aged 2 to 15 years (Peshkin and Landay[84a,b]); and 17.5% in allergic children aged 2 to 13 years, compared to 7.5% in nonallergic control children (Lima and Rocha[69]); these subjects had no contact with tobacco except in the most casual way, and they all showed multiple sensitization. Six of 24 allergic adult nonsmokers reacted to tobacco slightly, and all reacted moderately to extracts of ragweed, timothy, house dust, and feathers; of 17 allergic smokers in whom tobacco played no part in their clinical manifestations, 8 reacted to tobacco and all reacted to the other allergens (Trasoff, Blumstein and Marks[121]). Rosen[96] reviewed the office records of 200 consecutive patients with asthma, hay fever, perennial allergic rhinitis, eczema, urticaria, gastrointestinal allergy or migraine, and noted that 19% showed a good positive reaction to a mixed tobacco-leaf extract; of these 38 hyper-reactors, 4 had definite clinical entities which cleared up completely on stopping smoking. In a group examined by Fontana *et al.*[28] (personal communication), 15.8% of the 500 individuals skin-tested showed positive reactions to one or more of the tobacco extracts used; this figure was said to be in general agreement with those published for the incidence of allergy in the general population. Individuals with positive skin tests to tobacco related twice as much evidence of personal and familial allergy as the persons with negative skin tests. Sulzberger, Scholder and Feit[115] recorded that skin tests to tobacco were positive in 2 cases of migraine. Pipes[88] studied 370 consecutive allergic patients (smoking history not noted), 10% of whom gave a history of their respiratory allergy being precipitated or aggravated by tobacco smoke generated by some other person in the immediate vicinity; of the entire group of 370, 13% gave positive skin tests to tobacco-smoke extracts, while the same extract gave negative tests in 10 nonallergic controls. It is thus apparent that allergic individuals, children or adults, smokers or nonsmokers, are more prone to tobacco idiosyncrasy than nonallergic individuals, but similar findings would no doubt be obtained by testing with many other allergens: as put by Lima and Rocha[69], the predisposition of allergic children [and presumably of adults as well] to acquire sensitivity seems to explain why they had a higher incidence of positive reactions to tobacco. Peshkin and Landay[84a,b] analyzing the skin reactions of allergic children to tobacco and to pollen, found a striking parallelism between the two.

Several investigators have studied cutaneous sensitivity to tobacco in relation to *peptic ulcer*. Gray[36a,b] reported negative skin tests to tobacco extracts in 50 patients with duodenal ulcer associated wth tobacco smoking, as well as in 50 patients with functional gastric disturbances. Ehrenfeld and Sturtevant[24] determined tobacco sensitivity in 55 patients, all of whom were smokers except 3, with Roentgen diagnosis of peptic ulcer, and found that only 2 gave a marked positive reaction to the tobacco extract used. These latter authors[25] repeated their tests on a new series of 44 male patients with peptic ulcer using more concentrated tobacco extract, and obtained marked characteristic positive reactions in 4 cases. From the combined results of the 2 series, Ehrenfeld and Sturtevant concluded that it could not be proven that sensitivity to tobacco protein played any part in the pathogenesis of peptic ulcer, since in no case was it possible to demonstrate reagins, and in neither series were wheals obtained in significant proportions. Harkavy[43a] however, stated that the incidence of positive skin reactions to tobacco in patients with gastroduodenal ulcer was definitely larger than among normal people or control patients with other diseases; out of 60 such cases, 16 were sensitive to tobacco, and 11 of these had either a personal or family history of allergy (Harkavy[42a]).

In a group of miscellaneous disorders, skin tests with tobacco extract were positive in the one case of rheumatic heart disease tested; in 2 out of 3 cases of hypertension with precordial pain; in one case of precordial pain; and in one case of [? tobacco] neuritis (Sulzberger, Scholder and Feit[115]).

After reviewing papers on tests for tobacco sensitivity, Ross and Ivy[98] pointed out that a satisfactory type of test would be of great value to the physician as a guide to permitting limited, or forbidding the use of tobacco in patients with vascular disease. But Sulzberger[112] believed that both skin tests and reagin demonstrations with an allergen were of significance only in conjunction with, and in support of clinical evidence pointing to the same substance (tobacco). In view of his own findings, Chobot[18] wondered whether positive skin reactions to tobacco had any great significance or value; he concluded that great caution must be used in interpreting their value as a diagnostic test of any importance, and he too considered that a positive skin reaction could be interpreted as having significance only when the clinical test proved its importance. Lowell[73] pointed out that many apparently normal individuals, lacking any clinical evidence of allergic disease, exhibited skin sensitivity to intracutaneously injected allergens, and that skin sensitivity alone did not necessarily mean that the individual would have symptoms on exposure. On the other hand, absence of skin sensitivity in the opinion of Lowell and Schiller[74] was rarely, if ever associated with hay fever or asthma caused by pollens. Harkavy[48] agreed that, to establish clinical sensitivity to tobacco, it is imperative, just as in the case of positive skin reactions to any other allergenic substances, to correlate such reactions with the symptoms of the patient. In the case of tobacco, it must be possible to elicit clinical manifestations in the respective shock organ as a result of exposure, such as smoking or inhalation, followed by their disappearance upon its avoidance; only when this is achieved may the tobacco reaction be regarded as etiologically significant (Harkavy[48]).

Several investigators have pointed out, or implied, that skin tests should be made with extracts of tobacco

smoke as well as with extracts of leaf tobacco (Vaughan[123]; Pipes[88]; Rosen[95, 96]), although neither duplicates too well the actual situations encountered in human tobacco smoking (Rosen[95,96]). A basis for the latter difference might be that solubility of tobacco smoke in body fluids (saliva, bronchial secretions, and others) is not the same as that of tobacco smoke bubbled into the usual diluents (for example, Coca's fluid) used to prepare smoke extracts.

The existence of *tobacco allergy* appears to be indisputable, but its prevalence is difficult to determine, perhaps because of its protean manifestations. Smokers with positive skin tests to tobacco (16% of 79 individuals) were said to have presented 4 times more tobacco symptoms than those with negative skin tests (4% of 421 individuals), findings interpreted to show that certain tobacco effects should be regarded as allergic sensitizations rather than as poisonings (Fontana *et al.*[28], personal communication). Rosen[95] warned that skin testing with tobacco was not the final answer in deciding who is allergic to smoking; he considered as more important a "clinical entity" of signs and symptoms which cleared up completely on stopping smoking, and which could be reproduced by smoking (Rosen[96]). This author[95] stated that the incidence of tobacco allergy was quite low, but Maurer and Spain[77], after a brief review of the subject, concluded that tobacco might act as an allergen more frequently than suspected; frequently enough, according to Thomas[119], to justify its being included among the routine skin tests. It should be remembered, however, that allergic patients may complain of the mechanical irritation or toxic effect of tobacco smoke, even though they are not sensitized thereto (Vaughan[123]; Maurer and Spain[77]). So far as allergy is concerned, tobacco was said to play a more readily establishable role among individuals who work with tobacco than in those who smoke (Maurer and Spain[77]); thus, in a series of 260 tobacco-factory workers, 61 or 24% showed manifestations of allergy (Szentiványi, Filipp and Legeza[117]). Among the signs and symptoms said to be allergic manifestations of tobacco or smoking are: asthma, urticaria, angioneurotic edema, and contact dermatitis (see below); rhinitis (Rosen[95]; Heimann[53]); migraine (Rosen[95,96]; Harkavy[48]); leg pains (Rosen[95]); gastrointestinal symptoms (Vaughan[123]; Rosen[95]); cough (Rosen[95,96]); sore throat (Rosen[95]); and various cardiac disturbances, such as angina (Harkavy[47]; Fogg[27]; Rosen[95]; Harkavy[48]), extrasystoles (Smith[109]; Harkavy[48]), atrial fibrillation (Harkavy[48]), and tachycardia (Coca[19]; Richardson[92]; Harkavy[48]). Whether the acute disturbances in the cardiac mechanism are due to nicotine alone, or to a combined nicotine and tobacco idiosyncrasy, was in Harkavy's[48] view, the crucial question. Rosen[95] stated that smoking should be prohibited for anyone who has an allergy to tobacco smoke, that is, anyone who gets symptoms from smoking, whether gastrointestinal, vascular, or respiratory; Alexander[1] stated that tobacco was definitely contraindicated in nasal or respiratory allergy.

The older authors recognized *tobacco asthma;* according to Russo-Giliberti[99], the condition, although rare, was one of the forms in which chronic tobacco intoxication was manifested. Later authors considered that an incidence of inhalant allergy to tobacco or tobacco smoke of 1% was probably fairly representative of any large group of asthmatics (Brown[12]; Trasoff, Blumstein and Marks[121]; Vaughan[123]). However, a much larger proportion of asthmatics give low-grade positive endermal skin

reactions to tobacco extracts (Brown[2]; Vaughan[123]; for figures, see section on skin tests, above), apparently not specific in nature. Since tobacco contains proteins which may persist in its smoke after burning, it was claimed that smoking might be the direct cause of asthma (Thomas[119]; Lickint[68]), although there is no evidence that most forms of asthma have any etiologic relationship to smoking (Maier[76]). Smoking was said to be the major additive factor in a huge group of patients exhibiting signs of a major allergy (Petit[87]), and most authors have considered that smoking is contraindicated in asthma (Gutierrez Muro[39]; Schmidt[103]; Carryer *et al.*[15]; Peters *et al.*[86]; Petit[87]; Peters and Prickman[85]). It may be of interest to recall here that in former years tobacco was actually recommended as a remedy for relief of asthma on account of its antispasmodic effects (Sigmond[107]; Sée[106]); sometimes a mixture of tobacco and leaves of the thorn apple (*Datura stramonium*) was used in the form of so-called "asthma cigarettes" (Lickint[68]). That smoking may sometimes have a therapeutic effect in this condition was suggested by Barach *et al.*[3], who stated that when smoking produced cough, the expectoration of considerable amounts of bronchial secretion sometimes relieved the dyspnea of bronchial asthma; in the majority of patients tested, the smoking of 3 cigarettes in succession did not lead to increased bronchospasm, although it did so in some cases (Barach *et al.*[3]; Bickerman and Barach[8]). Reported cases of tobacco asthma are essentially similar, in that exposure to tobacco smoke was said to cause symptoms which were relieved upon removal from tobacco-smoke atmospheres; the patients also showed positive cutaneous reactions to tobacco (Russo-Giliberti[99]; Walker[125]; Hogner[56]; Jimenez-Diaz and Sanchez Cuenca[60]; Rosen and Levy[97]).

Vaughan[123] had a patient allergic to tobacco smoke who developed *urticaria* when smoking, and would manifest hives when she went into a restaurant where others were smoking, even though she was not. Rappaport and Hoffman[90] reported the case of a heavy cigarette smoker so sensitive that hives would break out if he entered a smoke-filled room or after shaking hands with a person who had shortly before smoked a cigarette. Rosen[95,96] mentioned a patient whose chronic urticaria ceased when he stopped smoking, and Harkavy[48] stated that tobacco allergy may be responsible for urticaria.

Although Vaughan[123] saw a young woman who developed *angioneurotic edema* of the lips whenever she touched them with any variety of cigarette, Silbert[108] reported what he claimed to be the first case of angioneurotic edema due to tobacco sensitivity reported in the literature. (Harkavy, in a personal communication to Silbert, stated he had seen at least 3 cases.) Silbert's patient, who had smoked 3 cigars and 10 cigarettes daily for years, also had thromboangiitis obliterans; the edema involved chiefly the lips and eyelids, but had occurred at times in other parts of the body; intradermal tests showed strong positive reactions to tobacco, ragweed, and timothy. A few weeks following discontinuance of smoking, the attacks of angioneurotic edema practically ceased, and complete recovery was eventually achieved.

Kulbs[64] listed *dermographism* as one of the characteristics of excessive use of tobacco, and presented an illustrative case history.

It is well known that tobacco may cause *contact dermatitis*; cases of dermatitis due to *tobacco smoke*, however, have not been reported (Schwartz[104]; Schwartz and Tulipan[105]). Some persons who develop dermatitis, especially

of the upper lip, when smoking cigarettes find that they can avoid this by using cigarette holders; others must discontinue smoking altogether (Vaughan[123]). Jehle[59] drew attention to the greater incidence of dermatitis among tobacco workers, compared to other workers. Dermatitis has often been described in tobacco workers (Pla[89]; Knowles[63]; Friedberger[31]; among others), in tobacco buyers (Rutledge[100]), and in tobacconists (Tommasi[120]). Panconesi[83] stressed the rarity of occupational tobacco dermatosis, in contrast to the statement of Pla[89] that, at one time or another, all workers in tobacco present lesions of the skin varying from mild erythema to tenacious eczema. According to Franchi[29], about 1.8% of women cigar workers contracted dermatoses of the hands; according to Samitz, Mori and Long[101], cases of hand eruptions constituted less than 0.04% of all patient visits to the dispensary of 3 cigar manufacturies. Cases of tobacco dermatitis have been reported by Knowles[63], Blaisdell[9], Karrenberg[61], Stauffer[110,111], Levi[67], Vero and Genovese[124], Milev[80], and Panconesi[83] among others. Tobacco dermatitis was the subject of a report published by the International Labour Office (Lancet 1, 308, 1934).

Panconesi[83] postulated an individual constitutional predisposition in patients with tobacco dermatosis, and thus an allergic pathogenesis rather than a primary toxic one or one related to repeated trauma. However, Franchi[29] and Samitz, Mori and Long[101] considered that the dermatitis of the fingers and hands in tobacco workers was not due to sensitization, since patch tests with tobacco leaves alone were negative, and an eosinophilia, which could be regarded as a sign of an allergic disease, was absent (Franchi[29]). Samitz, and co-workers considered that the condition was apparently produced, at least in the "examiners" in whom the greatest number of cases were seen, by the synergistic action of constant wetness of the hands plus the alkaline reaction of the toweling which the "examiners" constantly used as part of their work. Schwartz and Tulipan[105]; also Schwartz[104] considered that dermatitis among tobacco workers was mostly due to (1) the mechanical trauma involved in hand-stripping; (2) pastes used on cigars and boxes; (3) chemicals used in processing or flavoring agents; and (4) in rare cases, hypersensitivity [that is, idiosyncrasy] to tobacco, the actual irritant more likely being, however, traces of insecticides remaining on the tobacco or chemicals given off by the various curing processes, rather than to any natural constituent of the tobacco leaf. This latter view was concurred in by Vero and Genovese[124] who stated that no case of dermatitis due to either raw tobacco leaves or dry tobacco had been observed by them; they believed that the dermatitis reported by them in 3 cigar-makers had been caused by recently fermented tobacco. According to Milev[80], the susceptible worker was usually found to have abrasions of his skin which acted as sites of absorption. Among tobacco-associated but nontobacco causes of dermatitis, Rokstad[93] reported an outbreak of skin eruption caused by mites from tobacco, and Hering[54] described a form of skin rash developed by several workers while handling a particular lot of Turkish tobacco found to contain insects and insect feces.

According to Pla[89], the parts most affected in tobacco dermatitis were not in general those most exposed to the tobacco, but rather the various skin creases and folds of the body. Other reports, however, localized the lesions on the hands and fingers (Knowles[63]; Blaisdell[9]; Samitz, Mori

and Long[101]), or generally on the backs of the hands (report of the International Labour Office). In one case, the onset was sudden, 8 days after beginning work (Karrenberg[61]); in other instances, sudden, but only after many years of work in tobacco factories (Stauffer[110,111]; Vero and Genovese[124]). As in other plant idiosyncrasies, the susceptibility lasts for years, and the exposure needed to provoke an eruption is very slight (Knowles[63]; Blaisdell[9]). Milev[80] stated that the workers usually became immune to the condition on repeated exposure, but Rutledge[100] stated that practically the only cure for tobacco contact dermatitis was to get the patient away from tobacco.

Moncorps[81] unsuccessfully attempted desensitization in a case of eczema in a tobacco worker by means of intracutaneous injections of cigar-wrapper extract, and Stauffer[110,111] reported a similar case in which attempted desensitization by intramuscular injection of tobacco extract in increasing doses failed completely, although the injections were continued for a long time and the dose increased to the tolerance level. With respect to desensitization generally in tobacco allergy, in Vaughan's[123] experience, therapeutic desensitization was usually very satisfactory in the presence of true sensitization; but Rosen[96] stated that desensitization injections with tobacco extracts were of no help in treating tobacco allergies. Pipes[88] suggested that allergy to tobacco smoke could not be adequately controlled by hyposensitization with tobacco extract alone.

PART III. EFFECT OF NICOTINE, TOBACCO, AND SMOKING ON IMMUNE REACTIONS. Frei[30] claimed that increased resistance to infection through increased antibody production was obtained by suitable pharmacologic stimulation of the sympathetic or parasympathetic system by many autonomic poisons, including nicotine. A contrary view had been earlier expressed by Hubner[57], who noted a significant decrease in the effectiveness of salvarsan therapy during the post-World War I years, and blamed this on the immoderate use of tobacco, particularly of cigarettes, which was supposed to have paralyzed the natural defense mechanisms of the body. Experimentally, in rabbits poisoned with nicotine, Guardascione[38] found the antibody-producing response to the injection of ox erythrocytes to be decreased, the decrease becoming more evident as the amount of nicotine administered became greater.

Donzelli[22] acutely exposed 5 rabbits immunized against typhoid to the smoke from 5 to 7 cigarettes, and noted a drop in the agglutinin titer reaching a maximum about 6 hours after poisoning, with recovery in 24 to 48 hours. In another group of 4 immunized animals chronically exposed to the smoke of one cigarette a day for 20 days, lowering of the agglutinin titer was again observed, and to a degree even greater than in the acute experiments. In a third experiment, 4 rabbits were immunized during the course of chronic exposure to tobacco smoke, 2 others serving as controls; there was a definite retardation in the production of agglutinins in the exposed animals, and the final titer remained less than in the controls. *In vitro*, however, nicotine added in 0.5, 0.3 and 0.2% solution had no noteworthy effect on the agglutination of bacteria, either on the agglutinin in immune serum or on the agglutinability of the bacteria (Watanabe[127]).

Leva[66] studied the effect in rabbits of nicotine on the development of immune bodies (amboceptor) to intravenously-injected typhus culture. Five animals were given 1.5 to 2 mg. nico-

tine intravenously every day for 12 to 22 days (the dose of nicotine used regularly caused convulsions, followed by apathy and complete recovery), and typhus injections were made on the 5th, 8th or 17th day. Compared to 4 controls, the nicotine-injected animals showed a retardation in amboceptor production. Leva assumed that under the influence of nicotine, those cells whose function it was to produce antibodies suffered a lesion. In connection with Frei's[30] statement above, it is interesting to note that epinephrine had no effect on antibody production, although ephinephrine and nicotine both produced similar vascular changes in the aorta in experimental animals.

Mazzeo[79] studied the complementary power of rabbit serum in 3 animals after 10 daily injections of 1 to 2 mg./kg. nicotine; in 3 animals after one month of nicotine injection every third day; and in 3 controls. Acute nicotine poisoning was found to annul the complementary power; subacute moderate poisoning attenuated it. At the same time, acute nicotine poisoning markedly lowered the bactericidal power of the whole blood to staphylococcus and to *B. coli;* subacute moderate poisoning slightly lowered it. Using typhus bacilli as the test organism, Bruni[13] investigated the effect of acute and chronic exposure to cigarette smoke on phagocytosis in the rabbit; results were expressed in a phagocytic index, determined by the ratio: mean number of bacilli engulfed by each leukocyte after action of tobacco smoke/mean number before action of tobacco smoke. In 4 rabbits acutely poisoned by the smoke of 4 to 5 consecutive cigarettes, the phagocytic index after smoking varied from 0.48 to 0.70; 24 hours after smoking, it was 0.97 to 1.05; 48 hours afterwards, 0.97 to 1.03; thus, there was a marked diminution of phagocytosis through the acute action of tobacco smoke, but it was of brief duration, with return to normal after 24 hours. In 4 chronic experiments (1 cigarette a day for 15 to 49 days), there was a well defined drop in the index in each animal, the fall becoming more and more accentuated as the experiment progressed; final values for the phagocytic index ranged from 0.59 to 0.70. By further experimentation, Bruni found that cigarette smoke had no effect on the blood serum opsonin content, and he concluded that the decrease in phagocytosis must, by exclusion, be ascribed to a decreased vital activity of the leukocytes. In the *in vitro* system: leukocytes + blood serum + bacteria, with crossed tests made with serum and leukocytes from normal animals and animals poisoned by tobacco smoke, Bruni observed a diminished spontaneous phagocytic activity in animals subjected to tobacco smoke, which confirmed his conclusion that the diminution of phagocytosis observed in both acute and chronic tobacco-smoke poisoning resulted from direct action of the smoke on the leukocytes themselves. Using rabbits vaccinated with killed *Staphylococcus pyogenes aureus*, Mazzeo[78] confirmed that chronic nicotine administration (2 mg./kg. nicotine subcutaneously or by mouth, daily for 10 days, then every other day) sharply lowered the normal phagocytic index.

Lee[65] induced anaphylactic shock in 10 control female guinea pigs, then tested the effect of subcutaneous injection of 4.2 and 8.4 mg./kg. nicotine (according to Lee, one-tenth and one-fifth the female lethal dose) in 2 series of 6 animals each 30 minutes prior to the challenging injection. In the control series, 9 of 10 animals died; in the nicotine-treated series, all died, but the onset of symptoms was more delayed, the symptoms less, and the degree of emphysema was not uniformly as

severe as in the control series. Injection of 4.2 mg./kg. nicotine daily for 14 days increased the anaphylactic symptoms when the challenging injections were given. The author considered that the inhibitory effect of nicotine on anaphylaxis might be due to increased epinephrine secretion. Although daily doses of nicotine increased the anaphylactic symptoms when the challenging injections were given, the anaphylactic mortality was only 66.6%. Vallery-Radot, Mauric and Holtzer[122] gave intravenous injections of nicotine tartrate to 5 rabbits sensitized to horse serum. Following return of arterial pressure to normal, the rabbits were then given a shocking dose of horse serum intravenously, and a fall in blood pressure occurring in all animals was evidence of anaphylactic shock; the authors concluded that, in this species, nicotine had no inhibitory action on the appearance of anaphylactic shock.

Geiger and Alpers[34] investigated the mechanism of smooth muscle response to the antigen-antibody reaction and to nicotine. Contraction of sensitized guinea pig ileum owing to antigen (Schultz-Dale reaction), to serotonin, or to nicotine is prevented by low concentrations of alcohols and urethanes, suggesting a common step in the mechanism of the action of these 3 stimulants, probably conduction in nerve. Stimulation by antigen and by nicotine is prevented by structural analogs of serotonin (yohimbine, and others), and abolished by botulinum toxin. This suggests that antigen and nicotine may both act by liberating serotonin, as Fink[26] concluded in the case of antigen from studies on mouse uterus. However, ganglionic blocking agents do not prevent the Schultz-Dale reaction, while they are capable of inhibiting, often without completely blocking, the action of nicotine; this suggests a more complex mechanism of action for nicotine, that is, liberation of serotonin to act on *both* pre- and post-ganglionic fibers, instead of only on the post-ganglionic fibers as appears to be the case with the antigen-antibody reaction.

In view of the older belief that the antigen-antibody combination liberates histamine (or some pharmacologically similar H-substance) which is responsible for a number of the physiological and pathological manifestations of immediate type anaphylactic reactions, the following observations are included here: When isolated guinea pig intestine was treated with histamine, the tissue was found to be desensitized not only with respect to histamine, but also with respect to nicotine, or at least its sensitivity to these substances was greatly diminished (Werle and Effkemann[128]). Local application of a 5% solution of nicotine to the skin of rats produced a significant increase in the content of histamine in one animal, but had no effect in 2 others (Haas[40]); nicotine was said to cause histamine release (Haas[41]). Werle and Effkemann[128] studied the effect of smoking on the blood histamine level. In 10 male and 11 female nonsmokers, blood histamine averaged 0.04 gamma per ml. (range, 0.01 to 0.06), while in 18 male smokers and 11 female smokers, the average was 0.1 gamma and 0.081 gamma, respectively, (range, 0.05 to 0.20); in general, a high blood histamine content was found only in chronic smokers. Experiments on the effect of acute nicotine consumption on blood histamine showed that after inhaling the smoke of 6 cigarettes within an hour, a nonsmoker showed no increased blood histamine content. These authors, therefore, supposed that nicotine did not directly induce a flow of histamine into the blood, but that after prolonged heavy smoking

the body responded in this manner. No significant difference in the histamine content of plasma in smokers and nonsmokers was observed, and, since no leukocytosis was found, it was concluded that the increase in blood histamine represented an increased histamine content in the blood cells. On the hypothesis that thromboangiitis obliterans was a tobacco allergy, Businco and Visalli[14] studied the blood histamine levels of 6 patients with this disease; normal values were found in 2 cases, and in the others the values were moderately higher. The histaminase level in the blood of smokers showed no increase, as compared with nonsmokers (Werle and Effkemann[128]). Lounsbury and Oughterson[72] could find no correlation between the skin reaction of 100 unselected subjects to cured tobacco-leaf extract and to intracutaneous injection of dilute histamine solutions, from which they concluded that the positive skin reactions in hyper-reactors to tobacco were not the result of a simple capillary destruction due to the presence in the extracts of some nonspecific histamine-like substance.

This review of the immunological aspects of tobacco and smoking has been undertaken, not only to summarize what is currently known of the subject, but also to reveal the extent of the lacunae in our knowledge. It is scarcely an exaggeration to assert that, among all the fundamental biological studies of tobacco, the immunology of tobacco and its smoke has been one of the least adequately investigated in the laboratory, with respect to both quantity and quality of the experimental work. The relatively few and widely-scattered animal data available point up the pressing need for a classic immunological investigation of tobacco, not only in the interest of extending our scientific knowledge in this field, but also to serve as a sound immunological basis for arriving at valid clinical concepts of possible tobacco-linked disease conditions.

Although the immunology of tobacco appears at first glance to have been more extensively studied clinically than experimentally, virtually all reported clinical investigation has been limited to determinations of cutaneous sensitivity to tobacco extracts; and it must be regretfully admitted that much of this published work is equivocal, uncritical, and inadequately controlled. It should be added, however, that the question of unexceptionable controls for clinical material is indeed a difficult one. A well-designed and properly controlled program of clinical investigation of tobacco sensitivity in relation to disease is thus greatly to be desired. One may venture to suggest that future emphasis should be placed, not so much on the number of cases in each series, as on a thorough immunological investigation of each individual patient. And it should not be forgotten that the allergic subject represents, in a very real sense, the first part of an experiment in immunology already performed for the investigator. Osler once wrote: "As clinicians we observe the experiments of Nature;" and much of scientific interest and value can hardly fail to result from a careful, and critical, observation of natural and acquired idiosyncrasy to tobacco in man.

REFERENCES

(1) **Alexander, E. L.:** Virginia Med. Month., **75**, 161, 1948. (2) **Allen, E. V., Barker, N. W., and Hines, E. A., Jr.:** Peripheral Vascular Diseases. Philadelphia and London: W. B. Saunders Co., 1946.

(3) **Barach, A. L., Bickerman, H. A.,** and **Beck, G.:** Bull. New York Acad. Med., **28,** 353, 1952. (4) **Barksdale, E. E.:** Virginia Med. Month., **66,** 660, 1939. (5) Idem: (a) J. Am. Med. Assn., **115,** 672, 1940; (b) Virginia Med. Month., **67,** 393, 1940; (c) Med. Klin., (Berlin), **36,** 1314, 1940. (6) **Bergman, A.,** and **Rikles, H.:** Rev. Asoc. méd. argent., **49,** 1361, 1936. (7) **Bernot, E.:** Thèse D'Alger, Imbert Press, Algeria, 1938 (also cited by **Couturat, J.** *in* Presse Méd., **47,** 939, 1939). (8) **Bickerman, H. A.,** and **Barach, A. L.:** J. Lab. & Clin. Med., **43,** 455, 1954. (9) **Blaisdell, J. H.:** Urol. Cut. Rev., **28,** 664, 1924. (10) **Bowman, K. L.:** *Reported by* **Coca, A. F.,** Ann. Allergy, **5,** 458, 1947. (11) **Brooks, H.:** Bull. New York Acad. Med., **9,** 323, 1933. (12) **Brown, A.:** New York Med. J., **118,** 333, 1923. (13) **Bruni, A.:** Sperimentale, **85,** 523, 1931. (14) **Businco, L.,** and **Visalli, F.:** Policlinico, **48,** 16, 1941.

(15) **Carryer, H. M., Prickman, L. E., Maytum, C. K.,** and **Koelsche, G. A.:** J. Am. Med. Assn., **131,** 21, 1946. (16) **Chevallier, A.:** J. d. Chim. Med., pp. 326–333, 1839. (17) Idem: Bull. de l'Acad. de Med., **10,** 785, 1844–45. (18) **Chobot, R.:** J. Allergy, **6,** 383, 1935. (19) **Coca, A. F.:** Ann. Allergy, **5,** 458, 1947. (20) **Conner, L. A.:** Bull. New York Acad. Med., **9,** 327, 1933.

(21) **Deschiens, R.,** and **Poirier, M.:** C. rend. Soc. biol., **149,** 20, 1955. (22) **Donzelli, F.:** Gior. batt. e. immunol., **11,** 1012, 1933.

(23) Editorial: Med. Times, **45,** 171, 1917. (24) **Ehrenfeld, I.,** and **Sturtevant, M.:** Rev. Gastroenterol., **1,** 44, 1934. (25) Iidem: Am. J. Digest. Dis., **3,** 229, 1936.

(26) **Fink, M. A.:** Proc. Soc. Exper. Biol. & Med., **92,** 673, 1956. (27) **Fogg, A. H.:** J. Am. Med. Assn., **144,** 810, 1950. (28) **Fontana, V. J., Redisch, W., Nemir, R. L., Smith, M.,** and **Sulzberger, M. B.:** Communication to H. B. Haag, Medical College of Virginia. (29) **Franchi, F.:** Gior. ital. dermat. e. sif., **78,** 475, 1937. (30) **Frei, W.:** Schweiz. med. Wchnschr., **71,** 377, 1941. (31) **Friedberger:** München. med. Wchnschr., **73,** 1957, 1926. (32) **Friedlander, M., Silbert, S.,** and **Laskey, N.:** Proc. Soc. Exper. Biol. & Med., **34,** 156, 1936. (33) **Fukuda, Y.:** (a) Nippon J. clin. angiocardiol., **14,** 239, 1951; (b) Ibid., **15,** 28, 1951; (c) Ibid., **15,** 185, 1951.

(34) **Geiger, W. B.,** and **Alpers, H. S.:** Science, **125,** 1141, 1957. (35) **Gould, G. M.,** and **Pyle, W. L.:** Anomalies and Curiositis of Medicine. Philadelphia: W. B. Saunders Co., 1897, pp. 482–484. (36) **Gray, I.:** (a) Am. J. Surg., **7,** 489, 1929; (b) Tr. Am. Gastroenter. Assn., **32,** 489, 1930. (37) **Green, M. A.:** J. Invest. Dermat., **5,** 179, 1942. (38) **Guardascione, V.:** Rinasc. med., **19,** 39, 1942. (39) **Gutierrez Muro, F.:** Clín. lab. zaragoza., **25,** 217, 1934.

(40) **Haas, H. T. A.:** Arch. exper. Path. u. Pharmakol., **197,** 161, 1941. (41) Idem: Arch. exper. Path. u. Pharmakol., **205,** 170, 1948. (42) **Harkavy, J.:** (a) Bull. New York Acad. Med., **9,** 318, 1933; (b) Proc. Soc. Exper. Biol. & Med., **30,** 683, 1933. (43) Idem: (a) J. Allergy, **5,** 131, 1934; (b) Ibid., **6,** 56, 1934. (44) Idem: Proc. Soc. Exper. Biol. & Med., **36,** 381, 1937. (45) Idem: (a) J. Allergy, **9,** 275, 1938; (b) Ibid., **9,** 475, 1938. (46) Idem: J. Invest. Dermat., **2,** 257, 1939. (47) Idem: J. Mt. Sinai Hosp., **8,** 592, 1942. (48) Idem: Quart. Rev. Allergy, **11,** 189, 1957. (49) **Harkavy, J.,** and **Romanoff, A.:** J. Allergy, **6,** 62, 1934. (50) **Harkavy, J.,** and **Witebsky, E.:** Ibid., **6,** 437, 1935. (51) **Harkavy, J., Hebald, S.,** and **Silbert, S.:** Proc. Soc. Exper. Biol. & Med., **30,** 104, 1932–33. (52) **Hatcher, R. A.:** Am. J. Physiol., **11,** 17, 1904. (53) **Heimann, W. S.:** Bruxelles méd., **15,** 938, 1935. (54) **Hering, H.:** Derm. Wchnschr., **129,** 265, 1954. (55) **Högler, F.:** Wien. klin. Wchnschr., **54,** 1016, 1941. Also in: El Dia Méd. (Buenos Aires), **16,** 719, 1944. (56) **Hogner, R.:** Am. Med., **26,** 111, 1920. (57) **Hubner:** Derm. Wchnschr., **79,,** 1292, 1924.

(58) International Labour Office: Summarized in: Lancet, **1,** 308, 1934.

(59) **Jehle, L.:** Arch. unfallheilk., **3,** 364, 1899–1901. (60) **Jimenez-Diaz, C.,** and **Sanchez Cuenca, B.:** J. Allergy, **6,** 397, 1935.

(61) **Karrenberg, C. L.:** Derm. Ztschr., **52,** 30, 1928. (62) **Kaufman, K. L.:** Unpublished observations, Department of Pharmacology, Medical College of Virginia. (63) **Knowles, F. C.:** J. Cut. Dis., **31,** 11, 1913. (64) **Kulbs, F.:** Ztschr. klin. Med., **99,** 258, 1924.

(65) **Lee, Y. C.:** J. Severance Union M. Coll., **2,** 128, 1935. Also in: J. Chosen M. Assn., **25,** 1171, 1935, and Tr. Jap. Path. Soc., **25,** 201, 1935. (66) **Leva, J.:** Med. Klin., **3,** 450, 1907. (67) **Levi, I.:** Dermosifilografo., **12,** 1, 1937. (68) **Lickint, F.:** Arch. Pharm. Berl., **263,** 181, 1925. (69) **Lima, A. O.,** and **Rocha, G.:** Ann. Allergy, **7,**

528, 1949. (70) **Lopes de Faria, J.**: Estudo patológico da acao do tabaco desnicotinizado sôbre os vasos sanguineos do rato branco. Thesis. Univ. of Minas Gerais, 1943. (71) Idem: Rev. med. Brasil., **52**, 119, 1944. (72) **Lounsbury, J. B.**, and **Oughterson, A. W.**: Yale J. Biol. & Med., **7**, 305, 1935. (73) **Lowell, F. C.**: Allergy to Tobacco, *in*: **Wynder, E. L.** The Biologic Effects of Tobacco., Boston: Little, Brown & Co., 1955. (74) **Lowell, F. C.**, and **Schiller, I. W.**: J. Allergy, **19**, 100, 1948.

(75) **Maddock, W. G., Malcolm, R. L.**, and **Coller, F.**: Am. Heart J., **12**, 46, 1936. (76) **Maier, H. C.**: Smoking and lung cancer; bronchiectasis, asthma, bronchitis. reported in: **Coffin, G. J., Duryee, A. W., Maier, H. C., Pardee, H. E. B.**, and **Wynder, E. L.**: Bull. New York Acad. Med., **32**, 133, 1956. (77) **Maurer, M. L.**, and **Spain, W. C.**: J. Am. Geriatrics Soc., **2**, 278, 1954. (78) **Mazzeo, M.**: Boll. Soc. ital. biol. sper., **7**, 632, 1932. (79) Idem: Ibid., **17**, 136, 1942. (80) **Milev, T. I.**: Suvremenna Meditsina, **5**, 83, 1954. (81) **Moncorps**: Zbl. Haut Geschlkr., **30**, 804, 1929.

(82) **Newman, B. A.**: J. Am. Med. Assn., **111**, 25, 1928.

(83) **Panconesi, E.**: Rassegna di Dermat. e. di Sifil., **7**, 85, 1954. (84) **Peshkin, M. M.**, and **Landay, L. H.**: (a) J. Dis. Child., **57**, 1288, 1939; (b) *in* J. Allergy, **10**, 241. 1939. (85) **Peters, G. A.**, and **Prickman, L. E.**: Minnesota Med., **40**, 116, 120, 1957. (86) **Peters, G. A., Prickman, L. E., Koelsche, G. A.**, and **Carryer, H. M.**: Proc. Staff Meet., Mayo Clin., **27**, 329, 1952. (87) **Petit, P. T.**: South Med. J., **47**, 242. 1954. (88) **Pipes, D. M.**: Ann. Allergy, **3**, 277, 1945. (89) **Pla**: Languedoc méd.-chir., **14**, 87, 107, 131, 141, 155, 173, 1906; **15**, 34, 1907.

(90) **Rappaport, B. Z.**, and **Hoffman, M. M.**: J. Am. Med. Assn., **116**, 2656, 1941. (91) **Ratner, B.**: Reported in: **Peshkin, M. M.**, and **Landay, L. H.**: J. Dis. Child., **57**, 1288, 1939. (92) **Richardson, J. L.**: J. Med. Assn. Georgia, **45**, 11, 1956. (93) **Rokstad, I.**: Acta. dermat.-venereol., **24**, 113, 1944. (94) **Romanoff**, and **Rubin**: Cited by **Harkavy, J.**: J. Invest. Dermat., **2**, 257, 1939. (95) **Rosen, F. L.**: Mod. Med., **21**, 124, 1953. (96) Idem: J. Med. Soc. New Jersey, **51**, 109, 1954. (97) **Rosen, F. L.**, and **Levy, A.**: J. Am. Med. Assn., **144**, 620, 1950. (98) **Ross, P. H.**, and **Ivy, A. C.**: Quart. Bull. Northwestern Univ. Med. School, **20**, 424, 1946. (99) **Russo-Giliberti, A.**: Boll. soc. d'igiene Palermo, **1**, 323, 1886. (100) **Rutledge, W. U.**: Abstract of Discussion of: **Sulzberger, M. B.** J. Am. Med. Assn., **102**, 11, 1934.

(101) **Samitz, M. H., Mori, P.**, and **Long, C.-F.**: Indust. Med., **18**, 434, 1949. (102) **Schirren, C. G.**: Hautarzt., **2**, 324, 1951. (103) **Schmidt, R.**: Med. Klin., **34**, 665, 1938. (104) **Schwartz, L.**: Pub. Health Bull., **249**, 14, 1939. (105) **Schwartz, L.**, and **Tulipan, L.**: Occupational Diseases of the Skin. Philadelphia: Lea & Febiger, pp. 423–429, 1939. (106) **Sée**: Bull. gén de thérap., **77**, 385, 1869. (107) **Sigmond, G. G.**: Am. J. Med. Sci., **22**, 200, 1838. (108) **Silbert, S.**: J. Am. Med. Assn., **114**, 1442. 1940. (109) **Smith, A. L.**: Med. World, **61**, 297, 1943. (110) **Stauffer, H.**: Schweiz. med. Wchnschr., **10**, 1203, 1929. (111) Idem: Arch. Dermat. u. Syph., **162**, 517, 1930. (112) **Sulzberger, M. B.**: Bull. New York Acad. Med., **9**, 294, 1933. (113) Idem: (a) J. Allergy, **6**, 56, 1934; (b) J. Am. Med. Assn., **102**, 11, 1934. (114) **Sulzberger, M. B.**, and **Feit, E.**: J. Immunol., **24**, 425, 1933. (115) **Sulzberger, M. B., Scholder, B.**, and **Feit, E.**: J. Immunol., **24**, 85, 1933. (116) **Swineford, O., Jr.**, and **Radford, P. J.**: South Med. J., **41**, 667, 1948. (117) **Szentiványi, A., Filipp, G.**, and **Legeza, I.**: Orv. Hetil., **44**, 1414, 1951.

(118) **Thienes, C. H.**, and **Butt, E. M.**: Am. J. Med. Sci., **195**, 522, 1938. (119) **Thomas, W. S.**: Asthma, its Diagnosis and Treatment. New York: Paul B. Hoeber, Inc., pp. 130–131, 1928. (120) **Tommasi, L.**: Dermosifilografo., **6**, 57, 1931. (121) **Trasoff, A., Blumstein, G.**, and **Marks, M.**: J. Allergy, **7**, 250, 1936.

(122) **Vallery-Radot, P., Mauric, G.**, and **Holtzer, A.**: C. rend. Soc. biol., **138**, 336, 1944. (123) **Vaughan, W. T.**: Practice of Allergy. St. Louis: C. V. Mosby Co., pp. 675, 1015, 1939. (124) **Vero, F.**, and **Genovese, S.**: Arch. Dermat. & Syph., **43**, 257, 1941.

(125) **Walker, I. C.**: J. Med. Res., **36**, 423, 1917. (126) **Walzer, M.**: J. Allergy, **7**, 597, 1936. (127) **Watanabe, K.**: Tohoku J. Exper. Med., **24**, 111, 1934. (128) **Werle, E.**, and **Effkemann, G.**: Klin. Wchnschr., **19**, 1160, 1940. (129) **Westcott, F. H.**, and **Wright, I. S.**: J. Allergy, **9**, 555, 1938. (130) **Wood, W. L.**: Cited by **Theis, F. V.**, and **Freeland, M. R.**: Ann. Surg., **113**, 411, 1941. (131) **Wright, I. S.**, and **Moffat, D.**: J. Am. Med. Assn., **103**, 318, 1934.

RADIOLOGY

UNDER THE CHARGE OF

DAVID M. GOULD, M.D.
PROFESSOR OF RADIOLOGY, UNIVERSITY OF ARKANSAS MEDICAL CENTER
LITTLE ROCK, ARKANSAS

AND

JOHN G. MCAFEE, M.D.
ASSISTANT PROFESSOR OF RADIOLOGY, THE JOHNS HOPKINS UNIVERSITY AND HOSPITAL
BALTIMORE, MARYLAND

FIBROUS DYSPLASIA OF BONE

BY MARVIN L. DAVES, M.D.
ASSISTANT RADIOLOGIST, THE NATIONAL INSTITUTES OF HEALTH, BETHESDA, MARYLAND
FORMERLY RESIDENT, DEPARTMENT OF RADIOLOGY, THE JOHNS HOPKINS HOSPITAL

AND

JOHN H. YARDLEY, M.D.
RESIDENT PATHOLOGIST, THE JOHNS HOPKINS HOSPITAL AND INSTRUCTOR, DEPARTMENT OF PATHOLOGY, THE JOHNS HOPKINS UNIVERSITY

(From the Departments of Radiology and Pathology, The Johns Hopkins Hospital and University, Baltimore, Maryland)

FIBROUS dysplasia is a pyramid of diseases. The apex of the pyramid is the relatively rare Albright's syndrome, and its broad, indefinite base is a multitude of monostotic lesions. In 1937, Albright[2] and his co-workers crystallized the growing concept of an entity distinct from hyperparathyroidism which presented cyst-like bone lesions, cafe-au-lait spots and sexual precocity in females. In 1938 and 1942, Jaffe and Lichtenstein[41,43] extended this concept to include cases with only skeletal manifestations and supplied the name "fibrous dysplasia." So complete was their description of the clinical, roentgenographic, and pathologic findings in Albright's syndrome and polyostotic fibrous dysplasia that these diagnoses were thenceforth made with confidence. Thus the era of unnecessary parathyroid surgery came to an end. On the other hand, these authors only suggested the relationship of single osseous lesions to the parent disease and left to others the task of defining accurately the confines of monostotic fibrous dysplasia. In 1946, Schlumberger[55] stepped into this hiatus with an article entitled "Fibrous Dysplasia of Single Bones (Monostotic Fibrous Dysplasia)." Most authors have subsequently thought that his material supported the relationship between monostotic and polyostotic fibrous dysplasia. His own conclusion of no relationship has been usually disregarded. Confusion on this aspect still prevails, so that the diagnosis of fibrous dysplasia has become a "waste basket" for monostotic osseous lesions.

This review is undertaken in the

hope of shedding some light on the confusion surrounding monostotic fibrous dysplasia. Special attention will also be accorded other associated problems such as the complications encountered in classifying the numerous fibro-osseous lesions of the skull. Data and conclusions are based on our own experience and the literature which was available to us. A more extensive historical review is available elsewhere[51].

ETIOLOGY. The cause of fibrous dysplasia remains unknown. Jaffe and Lichtenstein's conception of a developmental abnormality of the bone forming mesenchyme is the most acceptable. Thannhauser's[65] contention that fibrous dysplasia is a variety of neurofibromatosis has inspired 2 very comprehensive rebuttals[1,33]. Other theories[3,8,46] have received less attention.

The problem of relating monostotic fibrous dysplasia to polyostotic fibrous dysplasia and Albright's syndrome etiologically is one of relating 2 unknowns. The monostotic lesion may be defined as any bony lesion which is commonly duplicated, roentgenographically and pathologically, in polyostotic fibrous dysplasia. The burden of proof is then on the one who says they are not related. Some authors, however, propose a traumatic basis for monostotic lesions. To Schlumberger, the predominant occurrence of the monostotic lesion in ribs is an argument in favor of trauma as the etiology; but, to the radiologist who commonly sees long-term follow-up examinations of rib fractures on routine chest films without observing the genesis of fibrous dysplasia, the opposite is true. Other arguments which Schlumberger advances are equally untenable.

CLINICAL MANIFESTATIONS. *Albright's Syndrome.* Albright's original description was of a syndrome characterized by osteitis fibrosa disseminata, areas of pigmentation and endocrine dysfunction with precocious puberty in females. He has since lamented the use of his name in connection with this syndrome and recommended substitution of Lichtenstein's term, "polyostotic fibrous dysplasia"[1]. The eponym, however, is widely accepted, and serves the function of separating the disease with extraskeletal manifestations from the purely osseous affection.

Cafe-au-lait spots are the commonest extraskeletal concomitant. They are distinguished by Albright from those of neurofibromatosis by having an irregular, "coast of Maine outline" as opposed to the smooth, "coast of California outline" found in the latter disease. In both diseases the cutaneous change evidently reflects an increase in the melanin content of the basal cell layer of the epidermis[34,45]. These spots show no tendency to follow the distribution of the bony lesions. Cases have even been reported in which the cutaneous involvement was confined almost exclusively to the side opposite to the skeletal involvement[54].

Sexual precocity is an impressive finding in females with Albright's syndrome. The menarche has been reported as early as the second day of life[24]. This precocity is a true one, because numerous females so afflicted have eventually had normal pregnancies. Lina Medina, the little Peruvian girl who became the youngest mother in the world at the age of 5[16], evidently had constitutional sexual precocity and not Albright's syndrome. Sexual precocity of mild degree has been reported in 3 males[17,69].

Skeletal precocity is less impressive but even more frequent than the early menarche and development of secondary sexual characteristics. Consequently females with Albright's syndrome are large for their age during adolescence but cease growing early and are be-

low average height as adults. Of the 3 male cases referred to above, only the one reported by Falconer and Cope[17] demonstrated a significant degree of early skeletal maturation. Jolly[37] has summed up our knowledge of the cause of precocity in patients with fibrous dysplasia by saying that it is as inexplicable as the etiology of the whole disease.

Thyroid dysfunction is frequently encountered, but its relation to the syndrome is unexplained. The range of congenital anomalies which have been reported in patients with fibrous dysplasia is wide and includes coarctation of the aorta, osteopoikilosis and arteriovenous fistula[47]. However, in a review of 140 cases of polyostotic disease, Vines[67] found the incidence of congenital anomalies no greater than in the general population. Our review of the literature leaves us with the impression that the mentality of these patients varies as does that of the general population. The high incidence of mental retardation first reported, in part reflected a lack of normal associations available to these physically handicapped children. Skeletal lesions are usually numerous in patients with Albright's syndrome but a few cases with minimal bony involvement have been reported[13].

Polyostotic Fibrous Dysplasia. As a rule, the clinical manifestations secondary to skeletal involvement are those produced by repeated fractures and the resulting deformities. In the skull, however, bony overgrowth may produce visible deformity and constrict the orbits and foramina causing exophthalmos and neurologic defects. We have seen one case (JHH #701499) in whom platybasia became a threat to life, but this patient also had Paget's disease. One case is reported in whom vertebral involvement resulted in paraplegia[64]. In cases with cranio-facial involvement, a dentist may first make the correct diagnosis. On the other hand, it is not uncommon for a patient to be asymptomatic despite rather extensive disease. The number of mild cases which remain unrecognized may be so great as to invalidate any statistics on the incidence of fibrous dysplasia.

Death may rarely occur as a result of superimposed infection in patients crippled by severe bony deformity. The disease does not always become inactive at puberty as has been suggested, and many patients present with their first symptoms in adult life. Orthopedic and cosmetic deformity, progressive blindness and contracted pelves are the main problems with which the clinician may have to deal.

In patients with extensive bony involvement, laboratory examination will frequently reveal an elevated serum alkaline phosphatase, occasionally a slightly elevated serum calcium, and rarely an increased urinary calcium excretion[3].

Monostotic Fibrous Dysplasia. These lesions are usually discovered accidentally, but may come to light as a result of pain, pathologic fracture or a noticeable mass.

Vines'[67] contention that 12% of the population have cafe-au-lait spots makes one suspect Schlumberger's[55] statement that none of his 67 patients with monostotic fibrous dysplasia had any abnormal pigmentation. Perhaps the basis of the discrepancy is a difference in definition of what constitutes abnormal pigmentations (stated by Vines but not by Schlumberger).

Fibrous dysplasia in its various forms has been reported in association with sarcoma in 11 instances[10,25,27,34,48,49,59,61] and with so-called adamantinoma of long bones in 5[5]. All of the cases, however, are not well documented, so that the malignant potentiality of fibrous

dysplasia remains in doubt. We are impressed by the paucity of bona fide malignant transformations reported in association with so common a bony lesion.

ROENTGENOGRAPHIC FEATURES. *Albright's Syndrome and Polyostotic Fibrous Dysplasia.* In the involved skeletal areas, the lesions tend to obliterate the medullary canal and thin the overlying cortex. If one observes a homogeneous, ground-glass medullary opacification which blends imperceptably with cortical bone and has no sharp margins (Fig. 3*D*), the diagnosis of fibrous dysplasia can be made with confidence. This distinctive homogeneous smudge, according to Hampton (in discussion of the paper by Bogart and Imler[7]), renders fibrous dysplasia the easiest possible roentgenologic diagnosis. Unfortunately, this appearance is not always present. On occasion, the cortex may be thinned, thickened or shaped like a candle flame. The medullary canal may demonstrate a single or multiloculated cystic appearance or a smudge resembling columns of smoke curling upward. Superimposed on, and perhaps responsible for, some of these lesions may be innumerable deformities wrought by repeated fracture. Thus, the variations in the roentgenographic findings are great. Nevertheless, the over-all picture is usually sufficiently characteristic to permit diagnosis even without the pathognomonic homogeneous smudge.

Fig. 1.—*(A)* (JHH #276369): A 4-year-old boy with polyostotic fibrous dysplasia. Deformity is minimal. There are areas of homogeneous smudging and a few cysts at the distal metaphysis of the tibia. *(B)* The same child at age 10, after numerous fractures. There is gross deformity with expansion and areas suggesting columns of smoke.

In some instances, we have seen the ground glass appearance become transformed into the "columns of smoke" picture as repeated fractures supervene (Fig. 1*A* and *B*).

The roentgenographic cysts are presumably a late feature because we have

encountered them much more frequently in adults than in children. This is in agreement with Hopf's observations[29], but the reverse has been reported by Sante[54]. The cysts sometimes reach grotesque proportions, whereas the ground glass lesion is virtually nondeforming. A multilocular appearance is sometimes due to thin areas of normal bone between cysts and sometimes due to coarse trabeculae partially traversing the cysts. Occasionally the cystic appearance is actually normal medulla between 2 adjacent dense smudges with concave borders.

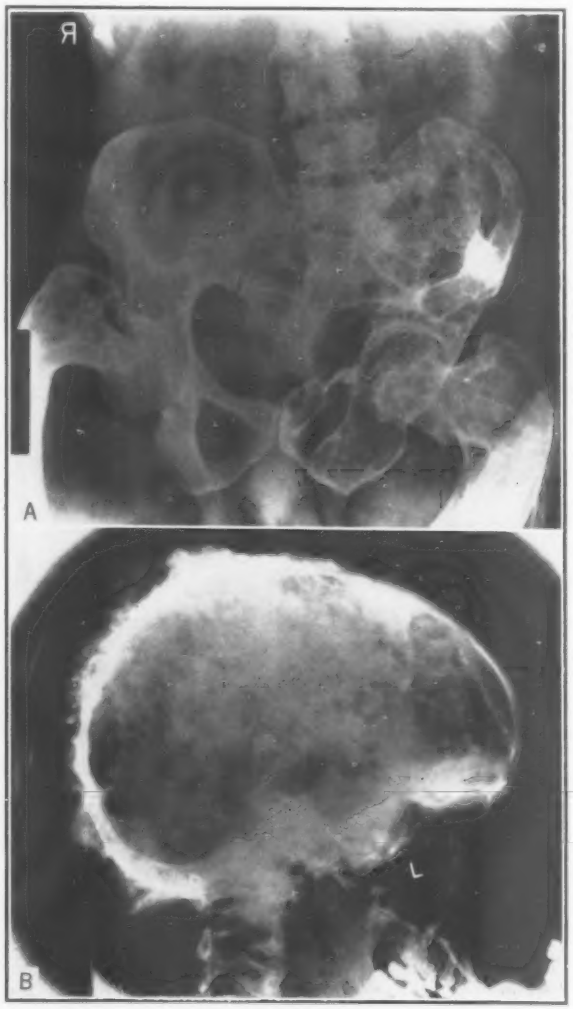

Fig. 2.—*(A)* (JHH #K67412): A 45-year-old man with predominantly left-sided involvement showing "shepherd's crook" deformity of both femora and large "cysts," particularly in the pelvis. Disease first noted at age 9. *(B)* Skull of the same patient, showing changes indistinguishable from Paget's disease.

The "candle flame" lesion is produced by an area of thickening of the cortex adjacent to a cyst, curving smoothly down to normal caliber beyond the cyst.

Extreme degrees of expansion, bowing, increase in length, distortion of bone, cystic formation and hyperostosis are generally associated with repeated fractures or infractions.

The distribution of the osseous lesions is extremely variable and erratic. In a given patient unilaterality of the lesions may be the most striking feature. Ribs are usually involved and occasionally undergo tremendous expansion not seen in other bones[18,19]. An equally dramatic finding is the collapse of the rib cage on one side producing a chest roentgenogram suggestive of an extensive thoracoplasty with regeneration of the resected ribs. The vertebrae are usually spared but may show cystic changes. Involvement of the pelvis is almost always cystic (Fig. 2A). In contrast, lesions in the diaphyses of the small bones of the extremities almost invariably appear as solid blocks having a homogeneous ground glass texture slightly less opaque than normal cortex. All graduations of the disease are seen in long bones, but the epiphyses are spared in children and seldom encroached upon in adults. No matter how extensive the osseous dysplasia, there will always be areas of absolutely normal bone in close proximity to areas of disease.

The skull is usually involved in polyostotic disease and will be discussed below.

Cranio-Facial Fibrous Dysplasia. Pugh[52], Windholz[71] and more recently Fries[19] have described these lesions in some detail. The base of the skull is characteristically expanded and of ground glass consistency but so much bone is seen tangentially in the roentgenograms that the result is a sclerotic appearance (Fig. 3, *A* and *C*). The calvarium is more likely to present a combination of sclerotic and lytic expansile deformity quite similar to Paget's disease but usually not so fuzzy (Fig. 4*A*). Purely cystic lesions are generally solitary, fairly small, and occur principally in the parietal area. The lesions usually have little regard for sutures, yet occasionally a single bone will be so uniformly hyperostotic as to afford an excellent demonstration of the anatomy of that bone. Facial lesions tend to be diffuse and to encroach on the sinuses, particularly the frontals and ethmoids (Fig. 3, *A* and *C*). Downward growths of the floor of the maxilla are common. Lesions in the facial bones may demonstrate all degrees of density but are usually less dense than those in the cranium. Mandibular lesions are likely to have very faint, coarsely speckled opacities (Fig. 5, *A* and *B*).

Monostotic Fibrous Dysplasia. All of the varieties of bony lesions encountered in the parent disease have been duplicated radiologically by the monostotic disease. Wells[70], Wyatt and Randall[72] and Zimmer[73] have given special attention to the roentgenographic features of monostotic fibrous dysplasia. They are of the opinion that the diagnosis can be made with confidence on a roentgenographic basis and give the following criteria: (1) A cystic-appearing area located eccentrically in the involved bone; (2) a smudge of increased density and a few coarse trabeculations within the cyst; and (3) opaque margination around the cyst which is sharp on the inner surface but, on the outside, fades off into the surrounding bone. We agree that a very high diagnostic batting average can be attained if the smudged appearance is present (Fig. 6*A*). In the absence of that sign, the lesions may be confused especially with the

equally benign nonossifying fibroma and small, inactive unicameral cyst[20].

PATHOLOGY. The typical lesion of fibrous dysplasia, whether it be polyostotic or monostatic, is seen grossly as gray to yellow, rubbery, frequently well-demarcated tissue lying within the medullary cavity of the involved bone. Sometimes the lesion shows a generalized reddish tinge from vascularization, or there may be a few frankly hemorrhagic foci. Cystic areas are occasionally encountered. A gritty texture involving a part or all of the dys-

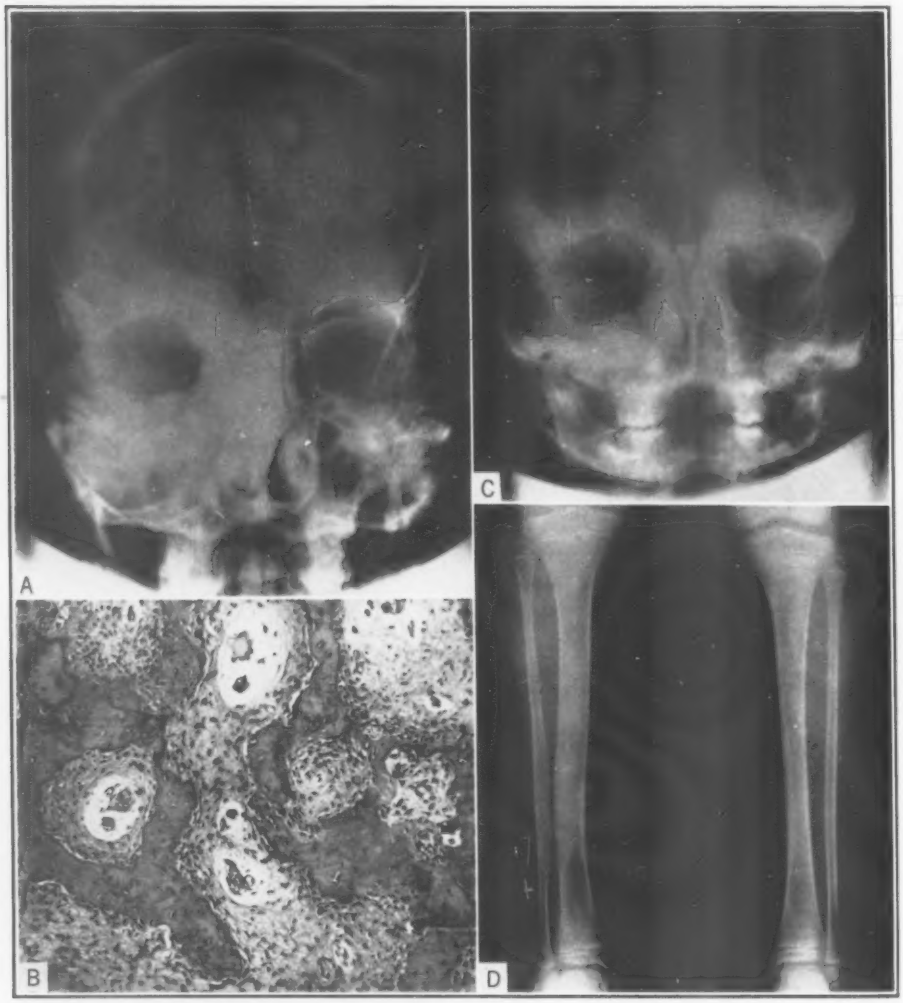

Fig. 3.—*(A)* (JHH #644222): A 19-year-old woman with cranio-facial fibrous dysplasia of the sclerotic type. There is constriction of the right orbit and filling in of the sinuses and nasal passage on that side. *(B)* The biopsy specimen shows thick, interconnecting masses of metaplastic osteoid. (Hematoxylin and eosin, × 75). *(C)* JHH #698199): An 8-year-old female with Albright's syndrome. Note the similarity to *(A)*. *(D)* Legs of the same child showing the pathognomonic smudge in the right tibia and fibula.

plastic process denotes the presence of calcified trabeculae. In extremely radiopaque lesions, the specimen is frankly bony, and the rubbery, fibrous component is less apparent. The periosteal surface is smooth and intact, except when trauma has been superimposed with its attendant callus formation. Cortical bone over the lesion is frequently reduced to a thin shell with a roughened "sand-pitted" inner surface. The degree and form of any over-all distortion of a given bone is related to location and extent of the intramedullary dysplasia.

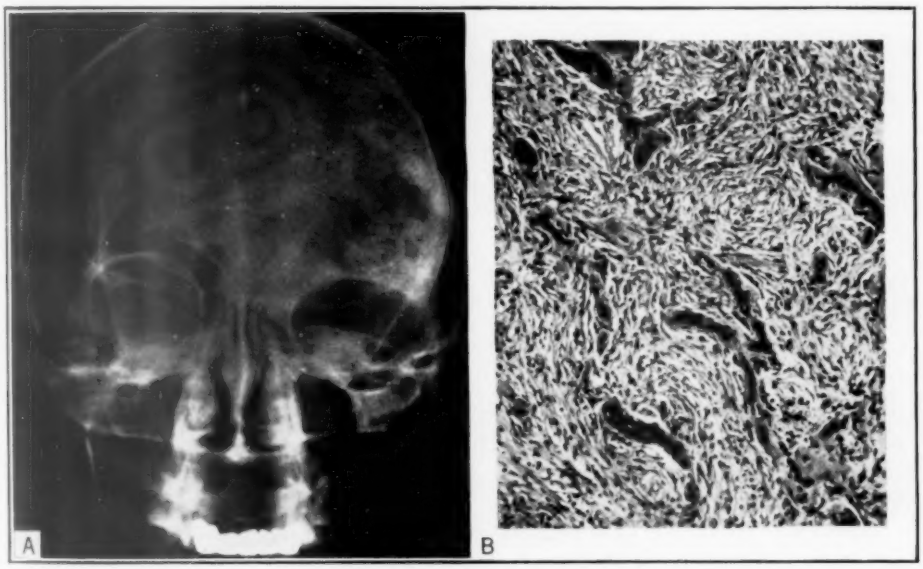

Fig. 4.—*(A)* (JHH #640319): A 64-year-old woman with cranio-facial fibrous dysplasia of the pagetoid type. There is constriction of the left orbit and occlusion of the left frontal sinus. *(B)* Histology of the same lesion, showing cellular fibrous tissue and associated bony trabeculae actively forming by metaplasia. (Hematoxylin and eosin, × 75).

Microscopically, the basic components of fibrous dysplasia are fibrous tissue and associated bony trabeculae. The fibrous tissue is ordinarily composed of spindle cells formed into strands and whorls, but in some specimens, the fibrous constituent consists of more loosely arranged cells, much like those seen in embryonic fibrous tissue. Still other lesions, or areas of the same lesion, will demonstrate a paucity of cells and show large amounts of collagen. In the "typical" case, the osteoid is scattered through the fibrous tissue as more or less isolated deposits that are irregular in shape, variable in size, and show lack of any special pattern of arrangement (Fig. 6*B*). Large areas may show no osseous component at all. The deposits may be very tiny, measuring as little as 20 to 40 micra in diameter; but at the other extreme, particularly in the radiopaque lesions seen most frequently in the skull, large, interconnecting trabeculae may be the major feature (Fig. 3*B*).

The osseous tissue in some lesions of the jaw is made up of unusually rounded trabeculae[6]. This has led to

the suggestion that it resembles osteocementum and that these jaw lesions may be more closely related to teeth than bone. Others[56] dispute this, stating that similar osseous spherules are seen in fibrous dysplasia elsewhere in the body (Fig. 6*B*).

A term frequently used in connection with fibrous dysplasia is "osteoid metaplasia." This aspect of the pathological picture is best studied in those lesions composed of multiple small bone trabeculae and abundant cellular fibrous tissue of the more primitive type (Fig. 4*B*). The metaplastic formation of osteoid is seen in its earliest stage as small deposits of osteoid that develop around individual connective

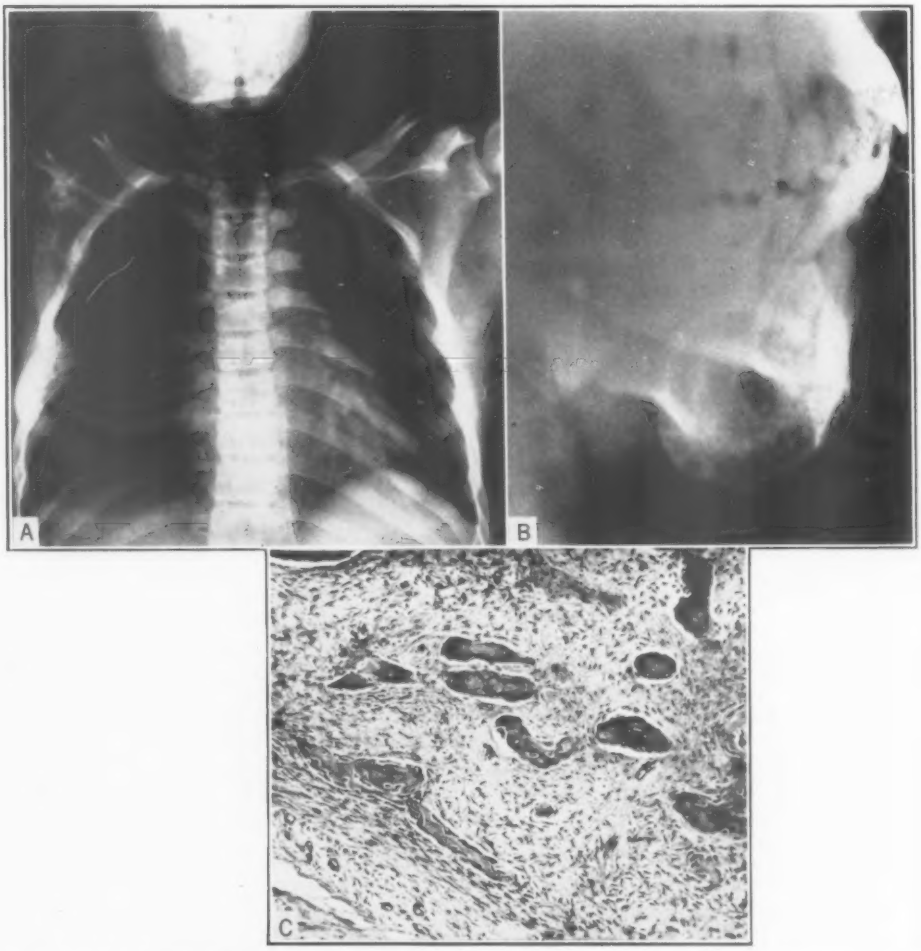

Fig. 5.—*(A)* (JHH #703684): A 15-year-old male with polyostotic fibrous dysplasia. The ribs and scapulae show cystic and sclerotic involvement. Note the unilocular, expansile deformity of the right side of the mandible. *(B)* Lateral view of the mandibular lesion. *(C)* A biopsy of the mandibular lesion is histologically indistinguishable from "ossifying fibroma." The trabeculae in the lower half are near the periosteum and show osteoclasts and osteoblasts on their borders. (Hematoxylin and eosin, × 75).

tissue cells. The connective tissue cells enlarge and become encased by the osteoid, and eventually the altered cells are trapped within the completely formed trabeculae as osteocytes[55]. Long fibers of collagen pass out into the fibrous tissue from the new trabeculae. This evidence of the common origin of the osteoid and collagen in fibrous dysplasia has been effectively illustrated by Valls[66] using the Rio Hortega stain. The similarity of osteoid metaplasia in fibrous dysplasia to embryonic preformation of membranous bone is striking.

Osteoblasts, in the sense of morphologically distinct, enlarged, rounded cells that line trabeculae, are not ordinarily encountered except at the margin of dysplastic areas where pre-

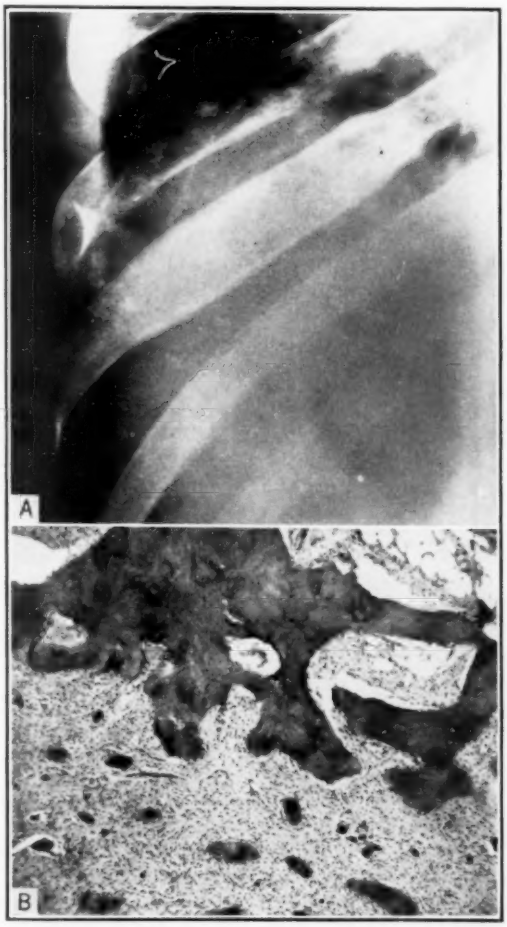

Fig. 6.—*(A)* (JHH #126514): A 49-year-old male with monostotic fibrous dysplasia in a rib showing slight expansion of the cortex and a homogeneous "smudged" appearance. *(B)* The lesion is seen at its margin. Its sharp demarcation and the small size of some osseous elements are apparent. Note that many trabeculae are in the form of spherules. (Hematoxylin and eosin, × 40).

existing bone and periosteum are being encroached upon by the growing fibrous tissue, or in lesions undergoing reactive effects following traumatic changes. The same factors also seem to govern the presence of osteoclasts, for they, too, are not characteristic of fibrous dysplasia (Fig. 5*C*).

Isolated giant cells may be observed rarely within the fibrous tissue, whereas greater numbers are chiefly noted around blood vessels and in areas where extravasation of blood has occurred[43]. Hence, trauma is implicated in their genesis also. Foam cells (cells containing lipoids) are infrequently seen in well-documented cases of fibrous dysplasia, and large numbers are unusual. Snapper[59], from the many foam cells he saw in his early cases, concluded that they were of primary importance in the pathogenesis of fibrous dysplasia. This he later retracted. Schlumberger[55] attaches special importance to foam cells as a common morphologic link between fibrous dysplasia and nonossifying fibroma. Since they are nonspecific and variable in occurrence in both fibrous dysplasia and nonossifying fibroma, we feel that foam cells should be given no special significance.

Occasional lesions from fibrous dysplasia will show nests of cartilage. Lichtenstein and Jaffe took this cartilage formation to be merely indicative of the potentiality of the fibrous tissue for forming both osseous and cartilaginous elements. Those authors also pointed out the similarity in some respects between polyostotic fibrous dysplasia and Ollier's disease; they stated that the presence of cartilage was but further evidence of the "kinship" between the 2 conditions. Since cartilage is a normal component of callus, it is also possible that the presence of cartilage in fibrous dysplasia is related to the traumatic alterations commonly sustained. Nevertheless, there have been a few cases of the polyostotic disease[63], including at least 2 cases[2,44,60] of Albright's syndrome with extensive endocrinopathy, that show large amounts of cartilage within lesions that were otherwise histologically fibrous dysplasia. Just how these cases fit into the total picture is not certain.

Early observers[59], before the recognition of fibrous dysplasia as a distinct entity, were sometimes puzzled by the realization that lesions in some cases of "von Recklinghausen's disease" that appeared cystic on Roentgen ray contained fibrous tissue with bony trabeculae on pathologic study. As was pointed out above, true cysts are the exception in fibrous dysplasia. One need compare the roentgenograms and pathology of only a few lesions to satisfy himself that relatively large amounts of metaplastic osteoid may be present without sufficient calcification to produce radiopacification. A ground glass or smudged roentgenographic appearance is caused by partial or helter-skelter calcification of trabeculae.

Although, in occasional foci, the trabeculae will show erratic cement lines that might cause confusion with Paget's disease, a true generalized mosaic pattern is not found in fibrous dysplasia.

As isolated histologic findings, fibrosis, osteoid metaplasia and osteosclerosis are, of course, in no way unique features of fibrous dysplasia. The failure to distinguish fibrous dysplasia from osteitis fibrosa cystica over a period of several decades lends special emphasis to this fact. Likewise, fibro-osseous changes are found in a number of other osseous conditions[15]. For example, fibrosis of bone is seen in agnogenic myeloid metaplasia, following exposure to a variety of poisons and in chronic internal exposure to irradiation. Metastatic tumor in a bone

will sometimes cause foci of fibrosis similar to fibrous dysplasia. Experimentally, fibro-osseous changes have been produced by injection of charcoal particles into a nutrient artery[40].

Changes brought on by trauma are particularly troublesome to the pathologist who is trying to diagnose a lesion where fibrous dysplasia is suspected. The fractures and infractions commonly sustained in many types of bone defect can alter a *bona fide* case of fibrous dysplasia in a way that makes the diagnosis suspect, or they can alter lesions of a different type (such as a cyst) in a manner that makes fibrous dysplasia a reasonable, though uncertain, possibility. Latent healing of an ordinary fracture can produce exuberant fibrotic callus that is misdiagnosed as fibrous dysplasia. Frequently, the surgeon is able to provide the pathologist only with small, nondescript fragments that make any interpretation hazardous.

NOMENCLATURE OF FIBRO-OSSEOUS LESIONS. *Cranio-Facial Fibrous Dysplasia.* In dealing with hyperostosis of the skull, when one eliminates Paget's disease, meningioma, metastatic disease, anemias, metabolic disturbances, hyperostosis frontalis interna and the osteo-dystrophies, one is still left with a group of fibro-osseous lesions. These may be almost completely fibrous or osseous, and may or may not have well-defined margins. This group has been subdivided into osteoma, fibrous osteoma (osteofibroma, fibro-osteoma), ossifying fibroma (central fibroma, osteogenic fibroma, odontogenic fibroma) and fibrous dysplasia, depending on the ratio of bony to fibrous tissue and the gross form of the lesion. With the exception of osteoma, we prefer to unify this group under the heading of cranio-facial fibrous dysplasia. This term circumvents the insignificant problem of whether fibrous dysplasia involving more than one cranial bone but confined to the skull is called monostotic or polyostotic. Hobeck[28] and Smith[58] encountered considerable difficulty in justifying their classification of these lesions as separate entities. Berger and Jaffe[6] and Georgiade[21] have illustrated the logic and simplicity of considering these lesions simply as fibrous dysplasia.

By "*osteoma*" we mean the eburnated and pedunculated growth usually seen protruding into the frontal and ethmoid sinuses but occasionally encountered elsewhere. While this lesion may be histologically identical to the other fibro-osseous lesions of the skull, unlike the rest, its distinctive gross and roentgenographic appearance is not a feature of polyostotic fibrous dysplasia or Albright's syndrome. Therefore, it must be considered a separate entity.

Monostotic Fibrous Dysplasia. We have defined monostotic fibrous dysplasia as any bone lesion which is duplicated in patients with polyostotic fibrous dysplasia or Albright's syndrome. As stated previously, the essential histopathology of the bony lesion is fibrous tissue in association with bony trabeculae. Thus, we have excluded osteoma because it has no gross or roentgenographic counterpart in the polyostotic disease. We have included fibrous osteoma and ossifying fibroma in the category of cranio-facial fibrous dysplasia because they are commonly duplicated in the parent disease. Many extracranial lesions have been called ossifying or osteogenic fibroma and these should also be considered monostotic fibrous dysplasia.

We must exclude 2 other lesions, which are sometimes considered monostotic fibrous dysplasia, because they are strictly fibrous and are not a feature of polyostotic fibrous dysplasia. These are nonossifying fibroma[35] (non-

osteogenic fibroma, fibro-xanthoma, xanthoma) and benign cortical defect[9], 2 lesions which are quite closely related. Hatcher[26] and Cunningham[11] have lumped both under the heading "metaphyseal fibrous defect" and Ponseti[50] has presented a case showing the genesis and exodus of 3 successive cortical defects which he feels proves that "non-ossifying fibroma" is a localized intermittent disturbance in the process of ossification. These 2 lesions do present the same microscopic picture (closely packed fibrous tissue and occasional nests of foam cells) and both occur primarily at the ends of long bones in an eccentric position. They differ clinically and roentgenographically in that nonossifying fibroma behaves like a tumor, while the cortical defect behaves like a disturbance in the process of ossification. The former expands the cortex, protrudes into the medullary canal, sometimes becomes quite large and does not regress spontaneously, while the latter appears in lieu of cortex, protrudes neither inward nor outward, remains quite small and is almost always obliterated by the remodeling process of the bone.

DIFFERENTIAL DIAGNOSIS. *Hyperparathyroidism.* The fact that 15 of the first 25 cases[17] finally recognized as fibrous dysplasia had parathyroid explorations is only of historical interest. There is no longer any reason to confuse fibrous dysplasia with hyperparathyroidism. The consistent chemical findings of hyperparathyroidism are not encountered in patients with fibrous dysplasia, and the skeletal roentgenograms of patients with hyperparathyroidism demonstrate generalized bony rarefaction of the malacic variety[12] with subperiosteal resorption, particularly in the phalanges and distal ends of the clavicles. These are changes which have no counterpart in fibrous dysplasia. Furthermore, for reasons as yet unexplained, the cystic lesions of hyperparathyroidism which led to the original confusion are being encountered less and less frequently.

Ollier's Disease. Because of the difference in the location and roentgenographic appearance of the bone lesions and of the histopathology involved, it seems to us that the difficulty in distinguishing fibrous dysplasia from multiple enchondromatosis is overrated. However, Pugh[53] has reported 3 cases of polyostotic fibrous dysplasia which did somewhat resemble Ollier's disease and has carefully outlined the distinguishing characteristics.

Neurofibromatosis. Among the differential points raised by Albright[1] and Jaffe[33] are the following: (1) Neurofibromata are not a feature of fibrous dysplasia. (2) The osseous lesions of neurofibromatosis are usually congenital malformations or deformities caused by slowly growing adjacent neurofibromata. (3) Cystic bony lesions resulting from intraosseous neurofibromata are rare. (4) The configuration of the cafe-au-lait spots differ in the 2 diseases. (5) Neurofibromatosis tends to be familial.

Eosinophilic Granulomata and Hand-Schüller-Christian Disease. The "punched-out" bony lesions of Hand-Schüller-Christian Disease[4] are easily distinguished from the lesions of fibrous dysplasia. The cutaneous changes found in the two diseases are quite different and the involvement of lungs and other viscera occasionally encountered in reticuloendotheliosis is not a feature of fibrous dysplasia. Solitary eosinophilic granulomata may occasionally require biopsy for diagnosis, but the lack of a condensed border or of any calcific density within the defect will usually distinguish them from monostotic fibrous dysplasia.

Paget's Disease. A small specimen

from an inactive area of Paget's disease devoid of marked osteoblastic and osteoclastic activity may closely resemble fibrous dysplasia and a few trabeculae in the latter sometimes contain cement lines forming a facsimile of the mosaic pattern of Paget's disease. However, given an adequate biopsy, the pathologist has no difficulty in identifying Paget's disease.

Radiologically, the distinction between Paget's disease and fibrous dysplasia can be quite difficult. Indeed, cranial fibrous dysplasia of the pagetoid type can be distinguished only by biopsy from Paget's disease, confined to the skull and without osteoporosis circumscripta (Fig. 2*B*). In polyostotic disease, the vertebrae will usually be most helpful. They are frequently involved in Paget's disease but rarely involved in fibrous dysplasia. In the former, they characteristically show increased radio-density, while in the latter, involvement is typically "cystic." The wedge-shaped advance of Paget's disease down the cortex of the long bone[22] is not duplicated by fibrous dysplasia and if metatarsals, metacarpals, or phalanges are involved, differentiation becomes easy. Paget's disease rarely involves these bones and then produces thick, irregular cortices and coarse trabeculation rather than the pathognomonic sign of fibrous dysplasia.

We would like to lament the tendency to dismiss casually Juvenile Paget's disease as a misdiagnosis for cases of fibrous dysplasia. A patient (JHH #168319), now 35 years old, has been followed at The Johns Hopkins Hospital for 20 years. Unfortunately, the early roentgenograms are missing, so the juvenile nature of the disease is not documented, but the age factor resulted in a misdiagnosis of fibrous dysplasia until the unfolding roentgenographic picture and repeated biopsies made it clear that this was truly a case of osteitis deformans. Wagner[68] reported a well-documented instance of advanced Paget's disease in a patient 18 years of age.

Leontiasis Ossea. Of the many terms which have been proposed with good intentions and perpetuated as "wastebasket" diagnoses, none has been so thoroughly abused as "leontiasis ossea." Windholtz[71] dispelled two-thirds of the confusion when he pointed out that most cases so diagnosed were either Paget's disease or fibrous dysplasia. A third and much smaller group he classified as Virchow's type. We feel that this group can be discarded and replaced with specific entities such as chronic osteomyelitis, diaphyseal dysplasia with cranial involvement[31] and metaphyseal dysplasia with cranial involvement[30], and we agree with Seils[57] who recommends abandoning the term leontiasis ossea completely. Unfortunately, the term will probably live on as a refuge for cranial hyperostoses which temporarily defy more meaningful classification. Insofar as the differential diagnosis of fibrous dysplasia is concerned, it is sufficient to be aware of the heterogeneous nature of leontiasis ossea and of the roentgenographic features of its component parts.

Meningioma en plaque. The clinical and roentgenographic features (including pneumography and angiography) of the spherical meningiomas[14] readily delineate them from fibrous dysplasia but meningioma en plaque presents a special problem[32]. Both cranial fibrous dysplasia and meningioma en plaque commonly produce sphenoid ridge and frontal hyperostosis, exophthalmos and optic changes secondary to progressive constriction of the optic nerve. In either condition, positive signs in pneumograms and angiograms may be minimal or absent.

It has been said that the 2 can be separated on the basis of age, the tumor occurring in adults and the dysplasia in children. However, our experience with cranio-facial fibrous dysplasia presenting in middle-aged patients has been too extensive to allow us to accept even this as a valid criterion. Perhaps scintoscanography will succeed in identifying meningioma en plaque where angiography and pneumography have failed.

Cherubism. This lesion must be considered in the differential, strictly because of nomenclature. Jones *et al.*[38] have used the name "familial fibrous dysplasia of the jaws" to indicate the disease known as cherubism which they have described so thoroughly. This is a nonosteogenic fibrous lesion which sometimes contains many giant cells, may be familial, and tends to regress spontaneously. All these features are foreign to fibrous dysplasia. Talley[62] and Gy. Frigyesi[23] have presented bona fide cases of fibrous dysplasia as familial fibrous dysplasia of the jaws (cherubism). We feel that the confusion can best be obviated by using the name "cherubism" which was originally suggested by Jones and is beautifully descriptive of the chubby face and "heavenward gaze" of children so affected.

Unicameral Bone Cysts. At its inception, the cyst grows adjacent to the epiphyseal plate, becomes rapidly larger, and finally migrates down the diaphysis as it becomes inactive[36] and as the epiphysis continues to form new bone. The classical lesion presents no difficulties in diagnosis. However, small lesions which are inactive when first seen roentgenographically may be indistinguishable from monostotic lesions of fibrous dysplasia that contain insufficient calcium to cause a "smudged" shadow. The surgeon makes the diagnosis when he cuts into a true cyst filled with yellow or brownish-yellow fluid. If the pathologist receives only a small scraping from the wall of the cyst, he may see nothing but fibrous tissue containing a few bony spicules that closely resemble fibrous dysplasia. Few lesions so beautifully illustrate the interdependence of radiologist, surgeon and pathologist.

Miscellaneous. Osteoid osteoma, giant cell tumor and aneurysmal bone cysts are lesions which are pathologically and roentgenographically distinctive and have received extensive consideration elsewhere[42]. On the other hand, the validity of "subperiosteal desmoid," a name supplied by Johnson for 4 cases reported by Kimmelstiel and Rapp[39], has not been established. These lesions may be related or identical to benign cortical defect, but fit none of the criteria of fibrous dysplasia.

Summary. 1. Fibrous dysplasia is a group of diseases comprising Albright's syndrome, polyostotic fibrous dysplasia, and monostotic fibrous dysplasia (including cranio-facial fibrous dysplasia).

2. Albright's syndrome is characterized by multiple skeletal lesions containing fibrous tissue associated with metaplastic bone formation, areas of cutaneous pigmentation and sexual or skeletal precocity.

3. Polyostotic fibrous dysplasia is characterized by the same multiple bony lesions with significant extraskeletal manifestations. Cafe-au-lait spots may or may not be present.

4. Cranio-facial and monostotic fibrous dysplasia include those isolated bony lesions that are identical, roentgenographically and pathologically, to lesions seen with polyostotic fibrous dysplasia. Since fibrous osteoma and ossifying fibroma are identical to lesions seen in the polyostotic disease, they are examples of fibrous dysplasia.

5. Because of one or more points of dissimilarity with fibrous dysplasia, nonossifying fibroma, benign cortical defects, and osteoma should be considered as distinct from fibrous dysplasia.

6. The name "cherubism" should be used to avoid the confusion caused by calling this lesion, which is apparently distinct from fibrous dysplasia, "familial fibrous dysplasia of the jaws."

REFERENCES

(1) **Albright, F.**: J. Clin. Endocrinol., **7**, 307, 1947. (2) **Albright, F., Butler, A. M., Hampton, A. O.**, and **Smith, P.**: New England J. Med., **216**, 727, 1937. (3) **Albright, F., Scoville, B.**, and **Sulkowitch, H. W.**: Endocrinology, **22**, 411, 1938. (4) **Avery, M. E., McAfee, J. G.**, and **Guild, H. G.**: Am. J. Med., **22**, 636, 1957.

(5) **Baker, P. L.**, and **Dockerty, M. B.**: J. Bone & Jt. Surg., **36–A**, 704, 1554. (6) **Berger, A.**, and **Jaffe, H. L.**: J. Oral Surg., **11**, 3, 1953. (7) **Bogart, F. B.**, and **Imler, A. E.**: Am. J. Roentgenol., **58**, 478, 1947. (8) **Bremer, J. L.**: A.M.A. Arch. Path., **32**, 200, 1941.

(9) **Caffey, J.**: Advances in Pediatrics, **7**, 15, 1955. (10) **Coley, B. L.**, and **Stewart, F. W.**: Ann. Surg., **121**, 872, 1945. (11) **Cunningham, J. B.**, and **Ackerman, L. V.**: J. Bone & Jt. Surg., **38–A**, 797, 1956.

(12) **Daves, M. L., Gould, D. M.**, and **Schultze, G.**: Radiology, **68**, 48, 1957. (13) **Dockerty, M. B., Ghormley, R. K., Kennedy, R. L. J.**, and **Pugh, D. G.**: A.M.A. Arch. Int. Med., **75**, 357, 1945.

(14) **Echlin, F.**: A.M.A. Arch. Surg., **28**, 357, 1934. (15) **Erf, L. A.**, and **Herbut, P. A.**: Ann. Int. Med., **21**, 863, 1944. (16) **Escomel, E.**: Presse méd., **47**, 875, 1939.

(17) **Falconer, M. A.**, and **Cope, C. L.**: Quart. J. Med., **11**, 121, 1942. (18) **Flickinger, F. M.**: J. Thor. Surg., **21**, 298, 1951. (19) **Fries, J. W.**: Am. J. Roentgenol., **77**, 71, 1957.

(20) **Garceau, G. J.**, and **Gregory, C. F.**: J. Bone & Jt. Surg., **36–A**, 267, 1954. (21) **Georgiade, N., Masters, F., Horton, C.**, and **Pickrell, K.**: J. Pediat., **46**, 36, 1955. (22) **Goldenberg, R. R.**: Bull. Hosp. Jt. Dis., **12**, 229, 1951. (23) **Gy. Frigyesi**: Fortschr. a. d. Geb. d. Rontgenstrahlen, **84**, 613, 1956.

(24) **Hackett, L. J.**, and **Christopherson, W. M.**: J. Pediat., **35**, 767, 1949. (25) **Hall, A., Bersack, S. R.**, and **Vitolo, R. E.**: J. Bone & Jt. Surg., **37–A**, 1019, 1955. (26) **Hatcher, C. H.**: Ann. Surg., **122**, 1016, 1945. (27) **Hobbs, A. A., Jr., Fisher, W. C.**, and **Beck, R. E.**: Am. J. Roentgenol., **76**, 320, 1956. (28) **Hoboek, A.**: Acta radiol., **36**, 97, 1951. (29) **Hopf, M.**: Radiol. clin., **18**, 129, 1945.

(30) **Jackson, W. P. U., Albright, F., Drewry, G., Hanelin, J.**, and **Rubin, M. I.**: A.M.A. Arch. Int. Med., **94**, 871, 1954. (31) **Jackson, W. P. U., Hanelin, J.**, and **Albright, F.**: A.M.A. Arch. Int. Med., **94**, 902, 1954. (32) **Jacobson, H. H.**, and **Vraa-Jensen, G.**: Acta radiol., **31**, 1, 1949. (33) **Jaffe, H. L.**: J. Mt. Sinai Hosp., **12**, 364, 1945. (34) Idem: Bull. New York Acad. Med., **22**, 588, 1946. (35) **Jaffe, H. L.**, and **Lichtenstein, L.**: Am. J. Path., **18**, 205, 1942. (36) Iidem: A.M.A. Arch. Surg., **44**, 1004, 1942. (37) **Jolly, H.**: Sexual Precocity. Springfield, Ill.: Charles C Thomas, p. 86, 1955. (38) **Jones, W. A., Gerrie, J.**, and **Pritchard, J.**: J. Bone & Jt. Surg., **32–B**, 334, 1950.

(39) **Kimmelstiel, P.**, and **Rapp, I.**: Bull. Hosp. Joint Dis., **12**, 286, 1951. (40) **Kistler, G. H.**: A.M.A. Arch. Surg., **29**, 589, 1934.

(41) **Lichtenstein, L.**: A.M.A. Arch. Surg., **36**, 874, 1938. (42) Idem: New England J. Med., **255**, 425, 1955. (43) **Lichtenstein, L.**, and **Jaffe, H. L.**: A.M.A. Arch. Path., **33**, 777, 1942.

(44) **McCune, D. J.**, and **Bruch, H.**: A.M.A. Am. J. Dis. Child., **54**, 806, 1937. (45) **McNairy, J. D.**, and **Montgomery, H.**: Arch. Dermat. & Syph., **51**, 384, 1945. (46) **Moehlig, R. C.**, and **Schreiber, F.**: Am. J. Roentgenol., **44**, 17, 1940.

(47) **Osgood, E. C.**: Am. J. Roentgenol., **56**, 174, 1946.

(48) **Perkinson, N. G.**, and **Higinbotham, N. L.**: Cancer, **8**, 396, 1955. (49) **Platt, H.**: Brit. J. Surg., **34**, 232, 1947. (50) **Ponseti, I. V.**, and **Friedman, B.**: J. Bone & Joint Surg., **31–A**, 582, 1949. (51) **Pritchard, J. E.**: Am. J. Med. Sci., **222**, 313, 1951. (52) **Pugh, D. G.**: Radiology, **44**, 548, 1945. (53) Idem: Am. J. Roentgenol., **71**, 632, 1954.

(54) **Sante, L. R., Bauer, W.,** and **O'Brien, R. M.:** Radiology, **51**, 676, 1948. (55) **Schlumberger, H. G.:** Mil. Surgeon, **99**, 504, 1946. (56) Idem: Am. J. Orthodon. & Oral Surg., **32**, 579, 1946. (57) **Seils, H.:** Fortschr a. d. Geb. d. Roentgenstrahlen, **80**, 738, 1954. (58) **Smith, A. G.,** and **Zavaleta, H.:** A.M.A. Arch. Path., **54**, 507, 1952. (59) **Snapper, I.:** Medical Clinics on Bone Diseases: A Text and Atlas. 2nd ed. London: Interscience Pub. Ltd., p. 209, 1949. (60) **Sternberg, W. H.,** and **Joseph, V.:** A.M.A. Am. J. Dis. Child., **63**, 748, 1942. (61) **Sutro, C. J.:** Bull. Hosp. Joint Dis., **12**, 217, 1951.

(62) **Talley, D. B.:** Oral Surg., **5**, 1012, 1952. (63) **Telford, E. D.:** Brit. J. Surg., **18**, 409, 1931. (64) **Teng, P., Gross, S. W.,** and **Newman, C. M.:** J. Neurosurg., **8**, 482, 1951. (65) **Thannhauser, S. J.:** Medicine, **23**, 105, 1944.

(66) **Valls, J.,** and **Schajowicz, F.:** J. Bone & Jt. Surg., **32–A**, 311, 1950. (67) **Vines, R. H.:** Arch. Dis. Child., **27**, 351, 1952.

(68) **Wagner, M.:** Wisconsin Med. J., **46**, 1098, 1947. (69) **Warrick, C. K.:** J. Bone & Jt. Surg., **31–B**, 175, 1949. (70) **Wells, P. O.:** Radiology, **52**, 642, 1949. (71) **Windholz, F.:** Am. J. Roentgenol., **58**, 51, 1947. (72) **Wyatt, G. M.,** and **Randall, W. S.:** Am. J. Roentgenol., **61**, 354, 1949.

(73) **Zimmer, J. F., Dahlin, D. C., Pugh, D. G.,** and **Clagett, O. T.:** J. Thor. Surg., **31**, 488, 1956.

SUMMARIO IN INTERLINGUA

(See page 505 for original article)

Le Concentrationes de Sulfamethoxypyridazina in Sanguine e Urina Post Administrationes Oral de Varie Dosages

Le concentration de sulfamethoxypyridazina (Kynex) in sanguine e urina esseva determinate in homines normal post unic doses oral de 4,0 e 3,0 g e in hospitalisate patientes adulte qui esseva sub tractamento secundo 5 differente planos de dosage. Le datos colligite es publicate como adjuta in le selection del appropriate plano de dosage in le utilisation prophylactic e therapeutic de iste nove e potentialmente importante sulfonamido.

In le tractamento del majoritate del infectiones susceptibile in adultos, un dose initial de 1,0 g sequite per 0,5 g omne 24 horas pare provider adequate concentrationes in le sanguine in le majoritate del casos. Un dose initial de 2,0 g es recommendate quando plus alte concentrationes in le sanguine es desirate tosto post le initiation del therapia, e doses diurne de 1,0 g es possibilemente requirite durante le prime dies del curso therapeutic si alte e ben-mantenite nivellos es requirite proque le infection in question es sever o proque illo es causate per organismos que es minus susceptibile al effecto del droga. Pro objectivos prophylactic contra infectiones streptococcal e contra febre rheumatic, le dose de 30 mg per kg de pesso corporee (*i.e.* 1,5 a 2,0 g pro adolescentes o adultos de pesos usque a 67 kg) un vice per septimana, de accordo con le recommendation de Lepper, Simon, e Marienfeld, pare esser rationabile e adequate.

Quando le tractamento con sulfamethoxypyridazina es continuate durante un septimana o plus, il es multo desirabile determinar le concentration del droga in le sanguine pro constatar si le absorption es adequate e non resulta in un accumulation excessive. Isto deveni un necessitate absolute quando le droga (o non importa qual altere sulfonamido) es usate in patientes con dysfunction renal suspecte o demonstrate.

BOOK REVIEWS AND NOTICES

ATLAS OF CLINICAL ENDOCRINOLOGY. By H. LISSER, A.B., M.D., Clinical Professor Emeritus of Medicine and Endocrinology, University of California School of Medicine, and ROBERTO F. ESCAMILLA, A.B., M.D., Clinical Professor of Medicine, University of California School of Medicine. Pp. 476; 148 ills., including 3 in color. St. Louis: C. V. Mosby Co., 1957. Price, $18.75.

THIS is an excellent pictorial representation of the problems seen by the clinical endocrinologist. Of particular value are the concise descriptions, including methods of diagnosis and therapy. The publisher is to be complimented on the format. As with all Atlases, this suffers from the fact that the cases presented are completely typical. This limits the value somewhat, so that the present volume cannot substitute for a good textbook. Nevertheless, it should be of extreme value to teachers of endocrinology. W. P.

PHARMACOLOGY AND ORAL THERAPEUTICS. By EDWARD C. DOBBS, B.S., D.D.S., F.A.C.D., Professor of Pharmacology and Therapeutics, Dental School, University of Maryland. 11th ed. Pp. 579; 28 ills. St. Louis: C. V. Mosby Co., 1956. Price, $9.00.

THIS edition is designed primarily as a teaching aid for dental students studying pharmacology; and for the practicing dentist as a reference book of newer drugs and preparations. I believe this aim has been very successfully achieved.

The book is divided into two sections. The first section discusses general pharmacology, with the drugs classified as to their chief sites of action. This section also includes a chapter on chemotherapy and antibiotics which is up to the minute. The newer local anesthetics are also fully reviewed. The second section is devoted to dental and oral therapeutics and it lists the drugs as to their dental uses, together with prescriptions useful in dental practice. A chapter on prescription writing should be of great help to the student as well as the practitioner, whose prescription experience is limited.

The book is an excellent compilation of drugs and treatments essential in the general practice of dentistry. In addition, it contains many references to the literature, making it a good text for reference purposes. H. H.

MODERN TRENDS IN ORTHOPEDICS (SECOND SERIES). Edited by SIR HARRY PLATT, LL.D., M.D., M.S., F.R.C.S., F.A.C.S., President, Royal College of Surgeons of England. Pp. 331; 226 ills. New York: Paul B. Hoeber, Inc., 1956. Price, $12.50.

THE editor is also the author of the paper on congenital dislocation of the hip. There are ten other subjects by leading orthopedists of Great Britain. The subjects are concerned with reconstructive surgery of the hip, peripheral nerve lesions, antibiotics in tuberculosis of bones and joints, muscle transplantation in poliomyelitis, internal derangements of the knee, traumatic paraplegia, Potts' paraplegia, displacement of upper femoral epiphysis, contractures of the hand and femoral neck fractures.

The authors have very aptly covered the topics which are so often encountered in everyday orthopedic practice. Latest therapies and therapy of the past are discussed, and the conclusions reached by each author are lucid. The book is well illustrated and should be on the shelf of orthopedic residents and all practicing orthopedic surgeons. A. S.

PHYSIOLOGIC PRINCIPLES OF SURGERY. Edited By LEO M. ZIMMERMAN, M.D., Professor and Chairman of the Department of Surgery, Chicago Medical School, and RACHMIEL LEVINE, M.D., Chairman, Department of Medicine, and Director, Department of Metabolic and Endocrine Research, Michael Reese Hospital, University of Chicago. Pp. 988; illustrated. Philadelphia and London: W. B. Saunders Co., 1957. Price, $15.00.

THE importance of applying the principles of physiology to the surgical patient is well recognized by all modern surgeons. However, the difficulties entailed in restudying the usual physiology texts and attempting to sort out important facts from large amounts of experimental detail present an almost insurmountable hurdle to the busy practicing surgeon. This task is greatly expedited by this book, which arranges a review in physiology into various surgical sections. Some of these, for example, are metabolic changes in injury, infections and antibiotics, transplantation of tissues, hemorrhage and shock, pain, congenital and acquired heart disease, and individual sections on each system. Multiple authors

have contributed, so that each section is authoritative and as timely as any text can be. It is doubtful that any physiology of any importance to a surgeon has been omitted. Actually this text is probably as complete as any current text in physiology, but differs only in arrangement of material, concise manner of presentation, and elimination of detail which would be valuable only to the experimental physiologist.

This book is highly recommended to all clinical physicians, particularly those engaged in the practice, teaching or study of surgery.

R. B.

KÖRPERLICHE UND GEISTIGE EIGNUNG ZUM FÜHREN VON KRAFTFAHRZEUGEN BEI HIRNVERLETZTEN. By DR. MED. ARTUR GROSSJOHANN. Pp. 84. Stuttgart: Georg Thieme Verlag, 1957 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, DM 7.50.

THIS monograph is based upon 5-years' experience in testing patients with chronic cerebral injuries. A battery of tests was applied among which an especially devised reaction apparatus and a tachistoscope proved useful. These two devices tested chiefly the patient's attention. The importance of testing the practical intelligence is emphasized. It is necessary to use several projective tests in order to ascertain characterological abnormalities. Mental abnormalities may impair the driving ability at least as much as purely neurological defects. Diffuse cerebral impairment seems to be more dangerous than focal lesions. Particularly frontal and temporal lesions cause inability to drive. One-third of the brain-injured tested were considered able to drive, one-third only under certain conditions (repeated retesting) and one-third was considered unable. The text will serve as an excellent introduction into these practically important problems.

E. S.

ATLAS DER GASTROENTEROLOGISCHEN CYTODIAGNOSTIK. By N. HENNING, M.D., Professor and Director University Clinic of Erlangen and S. WITTE, M.D., Assistant in Medicine. Pp. 103; 114 ills. Stuttgart: Georg Thieme Verlag, 1957 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, $9.50.

THIS monograph written in German contains a complete translation in English. Though short, it is comprehensive and deals with gastrointestinal cytodiagnosis from mouth to rectum. Of particular interest are the figures on duodenal contents demonstrating the cytopathology of a variety of diseases affecting the duodenum and biliary tree. The authors enumerate the techniques that are best applied in obtaining material for cytodiagnosis. They acquaint the reader with the difficulties, limitations and pitfalls of this technique but also stress its applicability. A variety of staining methods are suggested (including intravital fluorescent staining for tumor scanning) and the advantages of phase microscopy are beautifully represented. Cell types, numbers and arrangements are discussed in detail as are the variations in cytoplasm, nuclei and nucleoli. They point out that the large cell of pernicious anemia with enlarged nucleus, rich in chromatin, may also be observed in atrophic gastritis without pernicious anemia.

The most praiseworthy portion of this book is the Atlas. It contains beautiful photomicrographs of specimens of the entire intestinal tract including normals as well as those from inflammation, infection and a variety of tumors. It is regrettable that cost prevented the presentation of photomicrographs in color. This book is highly recommended to those with an interest in gastrointestinal cytodiagnosis.

S. L.

THE ORGANIZATION OF THE CEREBRAL CORTEX. By D. A. SHOLL, Department of Anatomy, University of London. Pp. 125; 24 ills. London: Methuen & Co., Ltd., and New York: John Wiley & Sons, Inc., 1956. Price, $4.25.

THIS is a beautifully written review of some of the recent studies on the cerebral cortex. It includes data obtained by anatomists, clinicians, physiologists, and psychologists, and correlates the information obtained in these different disciplines with each other and with a statistical approach advocated by the author.

In the first chapter methods of studying the cerebral cortex are reviewed and discussed. This is followed by very complete descriptions of the histology of the cerebral cortex, which includes both qualitative and quantitative data. Neuronal types, cortical lamination and cytoarchitectonic maps are considered in detail, as are the total volume of the cortex, the total number of neurons, and the density of neuronal packing.

Under the chapter heading "The Quantification of Neuronal Connectivity" the author describes the proportions of specific types of

neurons present in different parts of the cortex, the length and distribution of their dendrites, and the distribution of afferent fibers to different cortical zones. An interpretation of this histological analysis is included.

The studies of Pavlov, Lashley, and Hebb are reviewed in the chapter dealing with the mode of operation of the cerebral cortex. Also included in this section are discussions of Ashby's cybernetic methods and the studies of Walter on electroencephalography.

In the final chapter Professor Sholl presents some of the possible types of mathematical theory that might be useful in studying cortical organization. He believes that the probability theory and the related discipline of mathematical statistics are of value in the understanding of cortical activity.

The book is excellently organized. It should be read by everyone interested in the study of the nervous system. The photomicrographs are beautiful and the Bibliography, although selective, is very adequate. D. K.

THE DOCTOR, HIS PATIENT, AND THE ILLNESS. BY MICHAEL BALINT, M.D. Pp. 355. New York: International Universities Press, Inc., 1957. Price, $7.50.

THIS is a practical book growing out of a research project carried out at the Tavistock Clinic in England. The author and 14 general practitioners met once a week in groups of 8 or 10 and discussed experiences with patients. These discussions led by Dr. Balint, a psychiatrist, were focused around the emotional problems in the patient. The first thesis of the book was that some people who find it difficult to cope with their life problem resort to becoming ill. The doctor and his approach to illness has much to do with the organization of the illness. The book discusses psychotherapy by general practitioners and takes up such things as to when to give and when not to give reassurance and advice. It also deals with the time for starting psychotherapy and plans for terminating it. It is a readable book and contains an Index.

O. E.

YOUR CHILD OR MINE. (THE BRAIN-INJURED CHILD AND HIS HOPE). BY O. E. HOOD. Pp. 180. New York: Harper & Brothers, 1957. Price, $3.00.

THIS is the story of Mr. Hood's pioneer adventure in rehabilitating brain-injured children. It was as a psychologist that the author first came into contact with children, who were poorly understood and loved. While working as a teacher for abnormal children by day, he attended the Graduate School of Education at the University of Southern California at night and received his M.S. degree in 1948 and later continued as a member of the Doctorate program. In 1948 with a grant from the Tracy J. Putnam Foundation, he established a Psychological Clinic in Beverly Hills and until 1951, operated an experimental training school for abnormal children. In 1951, he opened his present institute for Child Study Training School, specializing in the problem of the brain-injured children.

Mr. Hood's book is simply written in lay language and is filled with the personal histories of the children who have come under his care. A surprising number of children, considered hopeless by all physicians who examined them, have returned to their homes, public schools and employment. This book will be of particular interest to the lay person who has a brain-injured child and who has a problem in his care. It will be of interest to the medical profession as a story of a pioneer experiment in rehabilitating brain-injured children. M. S.

PROCEEDINGS OF THE CONGRESS OF NEUROLOGICAL SURGEONS, 1955, LOS ANGELES, CALIFORNIA, CLINICAL NEUROSURGERY. VOL. 3. Pp. 261; 130 ills. Baltimore: Williams & Wilkins Co., 1957. Price, $10.00.

THIS monograph is presented with Dr. Carl W. Rand of Los Angeles as its honored guest. The contents are divided into six chapters. The first three chapters are devoted to lectures given to the Congress by Dr. Rand on pituitary tumors, histologic changes in the human brain following head injuries, and two cerebral complications of pregnancies respectively.

The fourth chapter is a Symposium on carotid artery thrombosis with an introduction and discussion by Dr. John E. Webster; Clinical Manifestations by Dr. J. M. Nielson; Pathological Manifestations by Dr. Cyril Courville; and Surgical Treatment by Dr. Lawrence W. Arnstein.

Chapter 5 is devoted to a Symposium on Consciousness with an introduction by Russell Meyers, M.D.; Psychological Aspects by Donald B. Lindsley, M.D.; Brain Stem Influences on Consciousness by H. W. Magoun, PH.D.; The Cerebral Cortex and Consciousness by Robert B. Livingston, M.D.; and Unconsciousness in Head Injuries by Dr.

John E. Webster. Included in this chapter are a series of questions and answers pertinent to this subject.

The final chapter is on a Symposium on Hypothermia and Hibernation in Neurosurgery. The introduction by Dr. William H. Sweet; The Clinical Aspects by Richard A. Koons, M.D. This chapter also includes many interesting questions and answers.

The monograph is completed by a list of the members of the Congress of Neurological Surgeons and an Index.

This monograph covers many neurological and neurosurgical subjects of wide interest and is stimulating and interesting reading.

M. S.

Diseases of the Nose, Throat and Ear. By Howard C. Ballenger, M.D., F.A.C.S., Professor Emeritus, Department of Otolaryngology, Northwestern University Medical School, and John J. Ballenger, B.S., M.S., M.D., Associate, Department of Otolaryngology, Northwestern University Medical School. 10th ed. Pp. 968; 550 ills., 11 plates in color. Philadelphia: Lea & Febiger, 1957. Price, $17.50.

This classic text has been widely used for 50 years and the 10th edition maintains the high standard of excellence by a thorough revision and the addition of much new material and numerous new and instructive illustrations. The physiology and functional tests of the vestibular labyrinth have been brought up-to-date and the chapters dealing with inflammatory diseases of the labyrinth and the surgical treatment of inflammatory diseases of the labyrinth have been completely rewritten. Entirely new chapters on allergy and Ménière's disease have been added. Malignant diseases of the nose, throat and ear are reconsidered in the light of recent advances in diagnosis and therapy. The chapters on peroral endoscopy, direct laryngoscopy, tracheobronchoscopy, esophagology and gastroscopy are concise, systematic and instructive. The authors have stressed diagnosis and treatment and thoroughly covered the clinical problems arising in the practice of otorhinolaryngology. This textbook will be of great value to the student, intern, resident, general practitioner and otolaryngologist. H. S.

Alcoholism: A Treatment Guide for General Practitioners. By Donald W. Hewitt, M.D., Chief Medical Advisor, Charity Alcoholic Rehabilitation Center, Los Angeles. Pp. 112. Philadelphia: Lea & Febiger, 1957. Price, $3.00.

The author of this book, who specializes in the treatment of alcoholism, indicates that he knows the alcoholic personality. He does not believe the problem of the alcoholic is entirely one of personality but he does not have any clear formulation of the part other factors play. His treatment approach is eclectic, giving the reader a choice of how much chemical treatment, how much religion, how much Antabuse, or how much psychotherapy he will use. The book might have had added value for the general practitioner (for whom it is intended) had the author indicated his preferred treatment technique and given reasons for its success instead of merely offering possible treatments. The list of references is sketchy. O. E.

Munro Kerr's Operative Obstetrics. By J. Chassar Moir, M.D., Nuffield Professor of Obstetrics and Gynaecology, University of Oxford. 6th ed. Pp. 1008; 403 ills. Baltimore: The Williams & Wilkins Company, 1956. Price, $20.00.

It is not easy to assume the authorship of a textbook which has been well-known and popular for almost 50 years. If the new author makes a sweeping revision of the material many will think he has made too many changes, whereas if he alters it only slightly he is likely to be accused of attempting to get by on the reputation of the original author. J. Chassar Moir has surmounted this problem admirably in his preparation of the 6th edition of Munro Kerr's Operative Obstetrics. He has retained much of the original material but has revised a considerable amount of it and has added personal comments and many excellent illustrative case histories from his own practice.

The title of this book is deceptive. In addition to the discussions of obstetric operations the author considers such problems as antenatal and intranatal care, anesthesia, asphyxia neonatorum, erythroblastosis fetalis and perinatal mortality. It could well be named "A Textbook of Abnormal Obstetrics." The material is divided into six sections: I) The Management of Normal Labor; II) Dystocia from Faults in the Forces; III) Dystocia from Faults in the Foetus; IV) Dystocia from Faults in the Parturient Canal; V) Obstetric Operations and VI) Injuries and Accidents to Mother and Child. Each section is remarkably complete; the rare and unusua

problems as well as the more common are considered.

The style is lucid and informal and the text is well illustrated with photographs, drawings and statistical tables. Many case histories and personal experiences of both Kerr and Moir are included. Specific diagnostic and treatment methods which the authors have developed from their great experience are presented in detail. Little which is impractical or experimental is included. The methods are, for the most part, those which are acceptable in this country as well as in England. The authors' consideration of the historical aspects of the material and their discussion of the evaluation of modern obstetric practices is most interesting and informative.

This book was prepared primarily for graduate students and practitioners rather than as a basic obstetric text. It contains so much important historical and clinical material and makes such delightful reading that it should be in the library of every obstetrician.

J. W.

NEW BOOKS

Medical Services for Rural Areas: The Tennessee Medical Foundation. By WILLIAM A. MASSIE. Pp. 68; 16 ills. Cambridge, Mass.: Published for The Commonwealth Fund by Harvard University Press, 1957. Price, $1.25.

THIS is the story of a program to improve the health service for a group of communities in rural Tennessee. It describes the work of The Tennessee Medical Foundation, incorporated in 1952 and aided by The Commonwealth Fund to assess and provide medical services in a rural area. This area, Pruden Valley, a secluded region in mountainous, northeastern Tennessee, has an area of about 100 square miles and a population of some 6000 persons, mostly coal miners and their families.

A survey of health needs taken during the summer of 1953 assessed the living and working conditions, medical, nutritional and dental problems of the inhabitants. Next came the establishment of community clinics, medical center and hospital: all in cooperation with the Tennessee State Medical Association.

This report describes the need, means and approach to solving an important public health problem such as is met in many rural areas of the United States today. F. R.

Toxemia of Pregnancy. Report of First Ross Obstetric Research Conference. Pp. 80; 10 ills. Columbus, Ohio: Ross Laboratories, 1957.

THIS is a report of the First Ross Obstetric Research Conference in which certain of the physiologic and pathologic changes accompanying the development of preeclampsia-eclampsia were discussed.

The material presented at the conference included discussions of the composition of the body in general and its various compartments, the distribution of electrolytes, renal function, pituitary and adrenal hormones, hypertension and pathologic changes in the kidney with preeclampsia.

There is little material which would be of great practical importance in the treatment of individual patients with preeclampsia but there is a tremendous amount of interesting information concerning the abnormal processes which produce the characteristic changes of toxemia.

J. W.

Hormones, Brain Function, and Behavior. Edited by HUDSON HOAGLAND, The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts. Pp. 257; 50 ills. New York: Academic Press, Inc., 1957. Price, $7.00.

THIS symposium on Hormones, Brain Function, and Behavior, is a "must" for workers in the field. Many of the papers are so technical that they would be of little interest for the general reader. Newer advances in the relationship between hormones and brain function are promising and interesting. W. P.

Strabismus: Diagnosis and Treatment. By BEULAH CUSHMAN, M.S., M.D., Associate Professor of Ophthalmology, Northwestern University Medical School. Pp. 208. Philadelphia: Lea & Febiger, 1956. Price, $6.00.

Mesenchymal Diseases in Childhood. Report of the 22nd Ross Pediatric Research Conference. Pp. 103; 25 ills. Columbus, Ohio: Ross Laboratories, 1957.

NEW EDITIONS

Expectant Motherhood. By NICHOLSON J. EASTMAN, M.D., Professor of Obstetrics, Johns Hopkins University. 3rd ed. Pp. 198; 13 ills. Boston: Little, Brown & Company, 1957. Price, $1.75.

IT is not often that a medical author can produce two "best sellers" particularly when one is directed toward physicians and the other toward their patients. Eastman has accomplished this with his "Williams' Obstetrics" and his enormously popular "Expectant Motherhood" which is now in the third edition. It is impossible to estimate how many thousands of women have read this book in its previous editions. Many obstetricians give each new prenatal patient a copy as part of his initial prenatal routine. Most women preserve their copies and lend

them to friends and it is one of the most popular books in city libraries.

The book is interestingly written and so complete that the patient and her husband should be able to find the answer to almost any question which may arise during the pregnancy. The material is presented in eleven chapters which begin with menstruation, conception and the development of the infant and conclude with a description of the care of the newborn baby. There are only a few illustrations but these are clear and illustrate important portions of the text: fertilization, intrauterine fetal development, uterine changes during labor, exercises, and others.

The author does an excellent job of giving specific advice and directions while at the same time making the instructions general enough to coincide with the beliefs and practice of most obstetricians. Most pregnant women and their obstetricians can learn much from this book. While it can never substitute completely for personal discussions of pregnancy and its problems with individual patients it can serve as a guide to which the patient can refer whenever a question arises between the periodic visits to her physician. J. W.

The Compleat Pediatrician. By W. C. Davison, M.D., Professor of Pediatrics, Duke University School of Medicine, and Jeana Davison Levinthal, M.D., Instructor in Pediatrics, University of Michigan School of Medicine. 7th ed. Durham, N.C.: Duke University Press, 1957. Price, $4.25.

The Compleat Pediatrician has become standard quick reference and guide for diagnostic and therapeutic information in the pediatric field. It is uniquely constructed to provide maximum information in the briefest possible space. Although it is scarcely serviceable as a means of introducing the undergraduate student to pediatrics, it has real advantages for the trained physician. This revision again brings the book up-to-date.

The senior author is fortunate in having elicited the help and interest of his daughter in the maintenance of *The Compleat Pediatrician* which has provided him not only an important part of his professional activities but has provided him an avocation as well. W. N.

THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

Published Monthly. Illustrated.

Subscription price: 1 year $10.00; 2 years $18.00.

(One year subscription to Canada, South and Central America and Spain, $10.36; all other countries $11.14.)

All foreign subscriptions payable in advance in United States dollars.

Business Correspondence: Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, Pa.

NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

Manuscripts intended for publication in The American Journal of the Medical Sciences, and correspondence should be sent to the Editor:

Dr. Richard A. Kern, Temple University School of Medicine, Philadelphia 40, Pa.

Articles are accepted for publication in The American Journal of the Medical Sciences exclusively, except in the case of subsequent publication in Society proceedings.

Manuscripts should be typewritten on one side only, of good quality paper and should be double spaced with liberal margins. The author's chief position and, when possible, the Department from which the work is produced should be indicated in the subtitle.

Illustrations accompanying articles should be numbered and have typed captions bearing corresponding numbers. For identification they should also have the author's name written on the margin or back. Line drawings and charts should be prepared with black India ink. Special arrangements can be made for printing color plates, elaborate tables or an excessive number of illustrations.

References should be numbered and at the end of the articles arranged alphabetically according to the name of the first author and should be complete, that is, author's name, journal, volume, page and year (in Arabic numbers).

Return Postage should accompany all manuscripts but will be returned to the author if the manuscript is accepted.

100 Reprints will be supplied gratis. Covers will be omitted on all articles. Orders of additional reprints will be supplied in multiples of 100. An order form showing cost of reprints is sent with proof. Individual reprints can usually be obtained by writing directly to the author.

THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

DECEMBER, 1957

ORIGINAL ARTICLES

ANGIOCARDIOGRAPHIC GLIMPSES OF CARDIAC PHYSIOLOGY*

By Melvin M. Figley, M.D.
ASSOCIATE PROFESSOR OF RADIOLOGY

(From the Department of Radiology, University of Michigan, Ann Arbor, Michigan)

To radiologists the world over, the name W. Edward Chamberlain brings to mind the figure of a man of seemingly boundless energy and enthusiasm, yet one possessed of independent and critical thought. Often the vigorous spokesman for his profession, Dr. Chamberlain is likewise a warm, kind friend to many of its members. In his lifetime the evolution of present-day radiology had depended on certain technical achievements. For some of these, precision control of radiographic processing (Chamberlain and Henny[11]), definition of human limitations at fluoroscopy and the need for image intensification (Chamberlain[8]), and electrokymography (Henny, Boone and Chamberlain[22,23]), Dr. Chamberlain and his staff must be given full credit. It is needless to eulogize him further before you, his students and colleagues who have the rare privilege of his stimulating association, but it is fitting that he know of our unfailing esteem which is expressed by the creation of this lectureship in his name.

It is equally fitting that on this first occasion we consider some aspect of cardiac physiology for this has been one of Dr. Chamberlain's perennial interests. As early as 1926, with Dr. Dock, he recorded the first accurate curves of heart border motion by the time-consuming analysis of films exposed with the Ruggles Roentgen cinematograph[9,10]. Twenty years later with the electrokymograph developed with Doctors Henny and Boone[22,23], similar curves could be obtained with exquisite ease and fidelity. More recently he has developed biplane stereoscopic serial filming equipment for cardiovascular filming[12], and now in conjunction with the Department of Physiology he and his department share in fascinating studies of circulatory physiology by cineradiography[15].

I have chosen to speak of glimpses of cardiac physiology by angiocardiography for with this method physiologic phenomena can be visually appreciated, if, however, incompletely. Angiocardiography is generally regarded as a morphologic examination, but it will be my theme to show that the simple

*First Annual W. Edward Chamberlain Lecture. Delivered at Temple University, Philadelphia, Pennsylvania, December 14, 1956.

expedient of coupling some timing device, such as the electrocardiogram, with the angiocardiogram provides one with physiologic information that is not otherwise available. Although the films are static images, only infrequently recorded, the timing record provides the continuity that allows perception of dynamic changes, an idea incidentally proposed by Ruggles in 1925[41], and more recently emphasized by Lind and Wegelius[48].

Observations may be made in this way about three related aspects of cardiac physiology: contraction of the chambers, the movement of blood and the action of valves. Few of these are of immediate practical importance, but they are of interest as they add to our understanding of heart action.

It may be asked, how representative are the angiocardiographic findings of the patient's usual hemodynamic status? Under optimum conditions venous angiocardiography disturbs the resting hemodynamics very little during the filming period. Selective angiocardiography by injection through catheter or needle within the heart, on the other hand, is less "physiologic." Without detracting from its value as a morphologic study, it is necessary to recognize the disturbing influences of the instruments themselves, the arrhythmias produced by injection and the general anesthesia which is usually required.

The proper intravenous injection of contrast material is painless, and in the usual amount of 1 ml./kg. of body weight is not large relative to the venous return. It is roughly equal to the ventricular stroke volume and enters the heart in 2 to 4 beats. Our experience shows that changes in heart rate or arrhythmia during filming are most unusual (Fig. 1). Such changes as do occur, brief bradycardia followed by tachycardia, begin with the peripheral distribution of the contrast material well after the useful films are obtained (Amundsen, Amundsen and Miller[1]). Furthermore, there is indication that right heart pressures are not necessarily altered by the injection. In normal dogs, Nordenström[35] and others, Rowe and colleagues[38] have found that injection directly into the right atrium produces no pressure changes that exceed those caused by respiration. Measurements in man on this point are lacking. Amundsen and associates have shown no alteration of systemic arterial pressure in man until the contrast material reaches the peripheral arteries (Amundsen, Amundsen and Miller[1]). Since the procedure can be carried out without anesthesia, with the patient either supine or sitting according to his cooperation, there is then reason to believe that it may be representative of his circulation at rest.

But this is not always true. Infants and young children may be inadequately sedated so they resist the restraints required for positioning. Injection and filming may then be carried out during struggling or crying. Apprehensive older children and adults may have tachycardia, hypertension and hyperpnea, or hypotension and nausea. Furthermore, in older patients filming is made with the breath held in inspiration but overzealous patients may unintentionally make a Valsalva effect with significant alteration of venous return. A painful injection in children may result in the same. It must be realized also that the contrast material is heavier than blood and will take a dependent position unless distributed by turbulence. Under difficult circumstances the examination is far from indicative of resting hemodynamics.

Then, of course, the response of an abnormal heart has to be considered

and it appears unlikely that there is no increase in right atrial pressure when the right heart chambers are dilated because of obstructive lesions or right ventricular hypertension. Contrast material, entering from the superior vena cava, often enters the hepatic veins under these conditions though it seldom does so normally. This suggests a limited capacity of the right atrium for augmented venous return. Presumably right atrial pressures are elevated during injection with the possibility that right to left shunting would be facilitated if the atrial septum were patent. Some pressure measurements under these conditions are needed. Noteworthy in this regard are Kjellberg's observations that injections directly in the vicinity of atrial or ventricular septal defects produce no right to left shunting unless there is complicating right ventricular hypertension (Kjellberg *et al.*[26]).

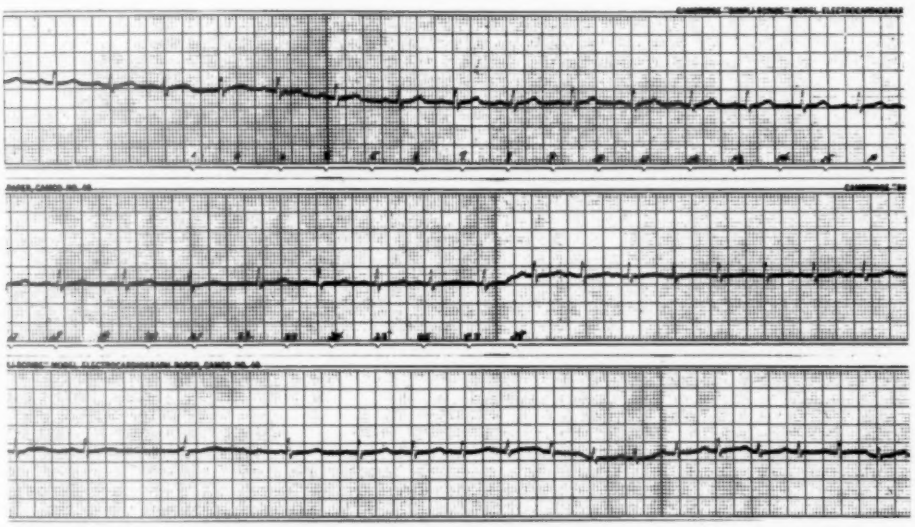

Fig. 1.—The electrocardiographic record during the angiocardiogram shown in Fig. 13. The very slight changes in rate during the filming, marked below, are not more than occur with respiration. Brief bradycardia, then tachycardia follow the filming.

NORMAL CHAMBER DYNAMICS. To appreciate fully the cyclic variations in heart morphology, it is necessary to relate them to other mechanical events and for this purpose phonocardiography or pressure measurement would be desirable. Electrocardiography, though not ideal, is widely used because of its convenience. Previous accounts of the relation of the angiocardiographic appearance and the electrocardiogram are quite limited (Lind and Wegelius[32]), and since modern filming equipment can record events of a few milliseconds duration it is necessary to relate mechanical and electrical events as precisely as possible. For this purpose the work of Cournand and associates[5,13], Leatham[29], and the monographs of Andersson[2], Rudhe[40], and Kjellberg and colleagues[26], all of which are in remarkable agreement, have been heavily relied upon in the preparation of the following account.

Right atrial systole begins roughly at the summit of the P wave (.065 sec. after its onset [Braunwald, Fishman and Cournand[5]]) and continues for .12–.18 sec. (Rudhe[40]). Atrial emptying begins .02 sec. later, according to electrokymographic observations (Rudhe[40]), and ceases by the closure of the tricuspid valve at the onset of isometric ventricular contraction. At this time, roughly coincident with the S wave, (.065 sec. [Braunwald, Fishman and Cournand[5]], .10–.11 sec. [Rudhe[40]] after the Q wave) the atrium has presumably reached its smallest dimensions. It then fills from the systemic veins to reach the maximum size, as judged by electrokymography, during isometric relaxation of the ventricle. The tricuspid valve then opens on the average of .14 sec. after the summit of the T wave (Rudhe[40]), the atrium empties rapidly into the ventricle and during the period of diastole fills somewhat again before the next atrial contraction. The atrial volume curve may then have a double wave form.

Left atrial time relationships are slightly different. Systole begins .02 sec. later (Andersson[2], and Lagerlöf and Werkö[28]) and ceases .02–.03 sec. earlier (Braunwald, Fishman and Cournand[5], and Leatham[29]), roughly at the peak of the R wave.

By angiocardiography (Figs. 2 and 3) it may be seen that the atria contract in all dimensions during systole, but corresponding border movements are not concentric. The posterolateral portion of the right atrium between the vena cavae and the dorsal portion of the left atrium between the pulmonary veins are relatively immobile (Fig. 9). Presumably venous attachments restrict the motion (Kjellberg *et al.*[26]). The atrial appendages contract concentrically (Kjellberg *et al.*[26]) and may empty quite completely whereas there is always some residual blood in the atria, somewhat less in the left than right. Localized narrowing of the vena cavae and pulmonary veins at their entrance occurs during atrial systole (Hedman, Lind and Wegelius[21], and Kjellberg and Olsson[27]). The competence of these sphincteric mechanisms in preventing reflux is unsettled yet.

Right ventricular emptying begins at the end of isometric contraction with the opening of the pulmonary valve which corresponds to the S wave or shortly thereafter (.08 sec. [Braunwald, Fishman and Cournand[5]], .10 sec. [Rudhe[40]] after the Q wave). There is some electrokymographic evidence that volume change in the right ventricle, as in the right atrium, is delayed approximately .02 sec. after the corresponding pressure change (Rudhe). Ventricular emptying is at first rapid, then slower and finally it ceases with the closure of the pulmonary valve at about the end of the T wave. Shortly thereafter the tricuspid valve opens and the ventricle fills, rapidly at first, then more slowly, and lastly to a variable degree during atrial contraction.

As with the atria, there is measurable asynchrony in ventricular dynamics. Left ventricular contraction is longer than on the right side (up to .05 sec.) but due to prolonged isometric contraction and relaxation the ejection period may be .06 sec. shorter (Braunwald, Fishman and Cournand[5]). Thus mitral valve closure at the beginning of isometric contraction precedes tricuspid valve closure (by .013 sec. [Braunwald, Fishman and Cournand[5]] or .02–.03 sec. [Leatham[29]]) but ejection does not begin until the aortic valve opens, about .115 sec. after the Q wave (Braunwald, Fishman and Cournand[5]), and some .035 sec. later than the pulmonary valve opens. Furthermore ejection ceases sooner on

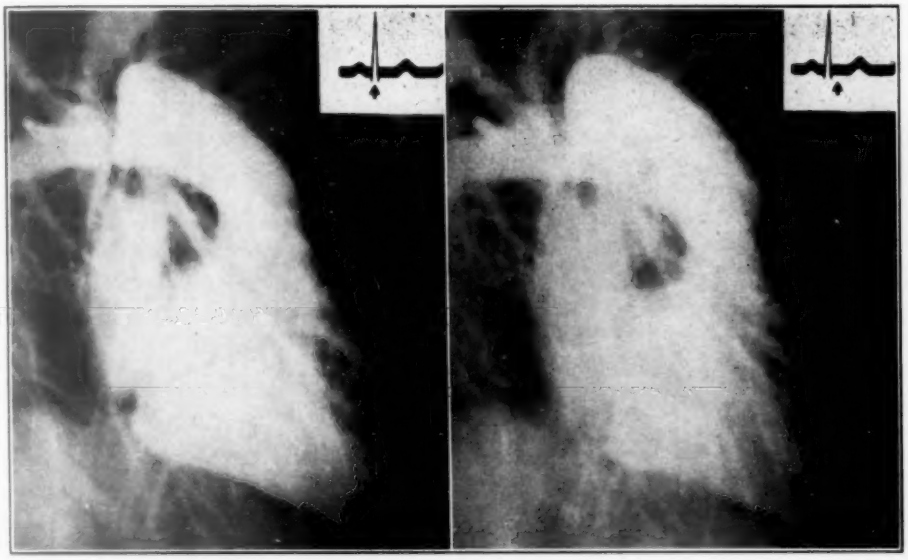

Fig. 2.—Normal right heart chambers seen in right anterior oblique projection, on the left near the completion of atrial systole and on the right during rapid ventricular ejection. Note in the latter that the atrium and its appendage are already well distended.

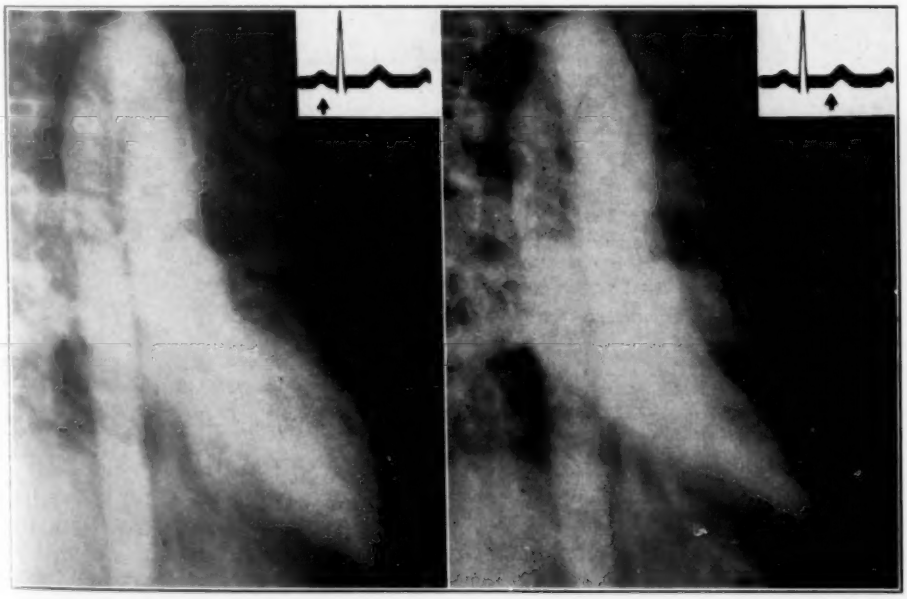

Fig. 3.—Normal left heart chambers. The same heart as in Fig. 2 in the same projection, shown on the left at about the onset of atrial systole and on the right when the left ventricle has completed most of its ejection. As is sometimes the case, the small normal left atrium is not seen well in this projection.

the left, somewhat before the end of the T wave, since aortic valve closure precedes pulmonary valve closure by .02–.03 sec. (Braunwald, Fishman and Cournand[5], Leatham[29]). Due to the long isometric relaxation period, however, left ventricular filling does not begin until .02 sec. after right ventricular filling (Andersson[2], and Rudhe[40]), or a little after the end of the T wave. The asynchrony of the two sides of the heart is more easily appreciated by the diagram in Fig. 4 taken from Braunwald, Fishman and Cournand[5].

By angiocardiography the ventricles are found to contract in all dimensions, more or less concentrically with residual blood conforming somewhat to the shape of the distended chamber. At times though only a small ovoid collection remains in the right ventricle and a cone-shaped one in the left below their respective infundibulae (Fig. 3). These latter structures are widest and less distinctly delineated during ventricular diastole. With systole they contract, becoming cylindrical, smooth walled canals, the right

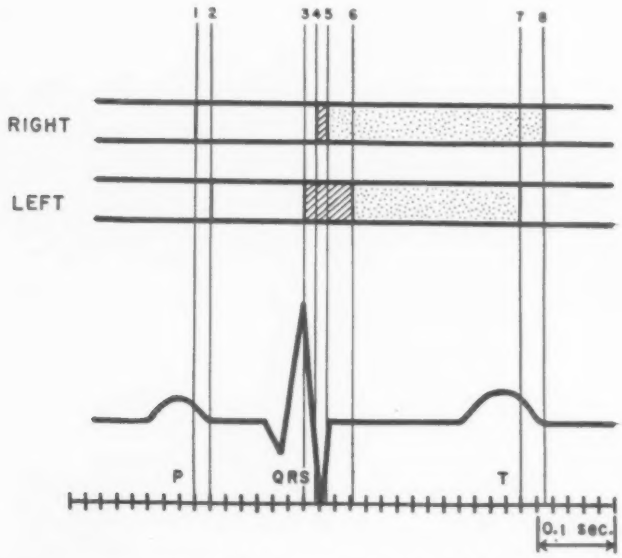

Fig. 4.—Diagrammatic representation of the average timing of electrical and mechanical events on both sides of the heart during atrial and ventricular systole in normal subjects.

(1) Onset of right atrial contraction.
(2) Onset of left atrial contraction.
(3) Onset of left ventricular contraction.
(4) Onset of right ventricular contraction.
(5) Onset of right ventricular ejection.
(6) Onset of left ventricular ejection.
(7) End of left ventricular ejection.
(8) End of right ventricular ejection.

The striped areas represent ventricular isometric contraction. The stippled areas represent ventricular ejection. Reproduced from Braunwald, Fishman and Cournand[5], Circulation, 4, 100, 1956 by permission of publisher, Grune & Stratton, Inc., New York.

longer than the left but both always filled with residual blood. According to current electrocardiographic concepts of the spread of the excitation wave, contraction begins later in these basal portions than in the sinus portions of the ventricle (Wiggers[49]).

A prominent feature of ventricular contraction is the associated movement of the valvular rings. With systole the semilunar valve rings move caudad and the atrioventricular rings show an even more pronounced apexward movement (Stauffer *et al.*[45]). Because of the latter, rather large reciprocal changes in volume can occur between the atria and ventricles with the result that much wider amplitudes of motion occur within the heart than on the surface (Fig. 8).

The observations described in the preceding sections were made in normal children and adults under various circumstances: at rest sitting, lying and at thoracotomy. They do not necessarily apply to infants, or to diseased hearts, and of course the time intervals vary with changes in heart rate. Nevertheless they can serve as a guide for the detection of gross alterations in heart chamber contraction.

ALTERATIONS IN CHAMBER CONTRACTION. It is possible with present-day rapid biplane angiocardiography to measure ventricular volumes (Dodge and Tanenbaum[14]) and it is conceivable that in the future end diastolic volume, systolic residual blood, stroke volume and cardiac output will be computed and related by this method. But at the moment with normal standards lacking, only gross alterations in ventricular contraction can be recognized by angiocardiography.

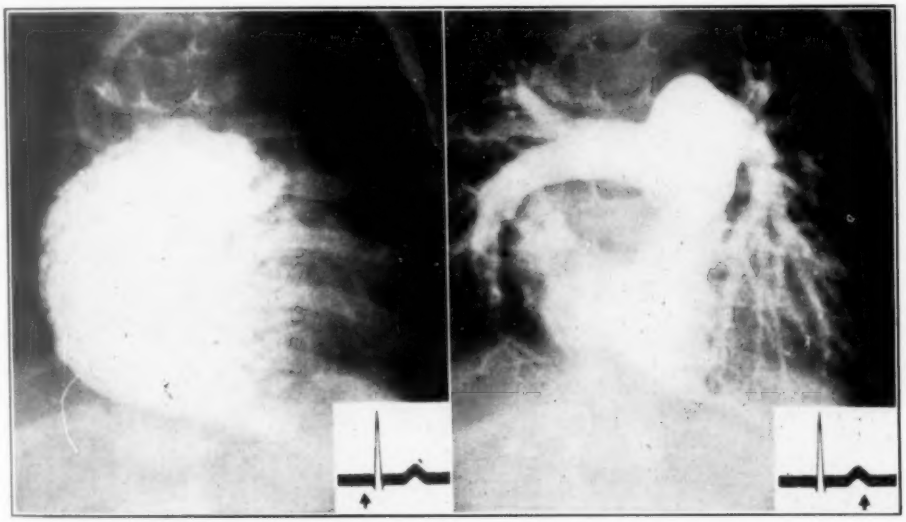

Fig. 5.—Complete A–V block, frontal projection. *Left*, the right ventricle at the end of its long diastole during which there were two atrial contractions. At this instant, it is larger than normal and forms the right border of the heart shadow. *Right*, at the end of the ensuing ventricular contraction, the ventricle has the usual systolic residual. The very large stroke volume was sufficient to fill most of the pulmonary arterial bed. Note the large excursion of the pulmonary valve plane.

Complete A–V heart block provides an example of exaggerated stroke volume. As shown in Fig. 5 the right ventricle is more than usually distended in diastole and normally emptied by the ensuing ventricular contraction. A large amplitude caudad movement of the pulmonary valve ring is also shown. By more rapid filming, alterations in atrial dynamics in this condition have been shown by Lind, Wegelius and Lichtenstein[33].

The manner of contraction of the infundibulum of the right ventricle is of practical importance in regard to the recognition of pulmonary stenosis. Hypertrophy of the right ventricle in

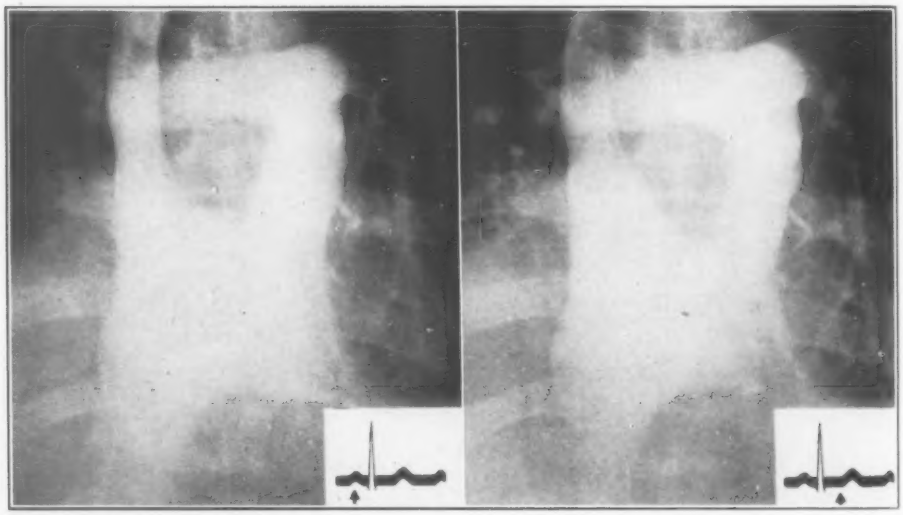

Fig. 6.—Constrictive pericarditis, frontal projection. Pleural fluid lies against the right heart border. *Left*, just prior to atrial systole; *right*, during rapid ventricular ejection. The right atrial margin is of unvarying contour, slightly concave from the right instead of convex. The ventricular stroke volume is almost certainly less than normal.

Constrictive pericarditis is distinguished by a low fixed stroke volume, the cardiac output varying with the heart rate (Sawyer *et al.*[42]). Occasionally this small stroke volume can be detected by angiocardiography (Fig. 6). Features more regularly and directly demonstrated are deformity and rigidity of the right atrial wall (Figley and Bagshaw[19]). The normal convexity and variability of the lateral margin of this chamber are lacking,

response to valvular pulmonic stenosis involves the infundibulum as well as the sinus portion of the ventricle with the effect that systolic contraction of this area may result in a channel much narrower than the normal. This might be regarded as functionally important stenosis but angiocardiographic observations indicate this is seldom if ever the case as the narrowing is pronounced only at the end of ventricular systole and the lumen then is virtually

always larger than the hole in the valve (Kjellberg *et al.*[26], and Campeti[7]). On the other hand, should the infundibulum be narrowed in diastole, as in Fig. 7, one may be certain that infundibular stenosis is present. In such cases systolic contraction may close the lumen completely. The reliable recognition of infundibular stenosis by angiocardiography depends upon having films exposed in ventricular diastole[14,30] (Kjellberg *et al*[26], and Figley[16]).

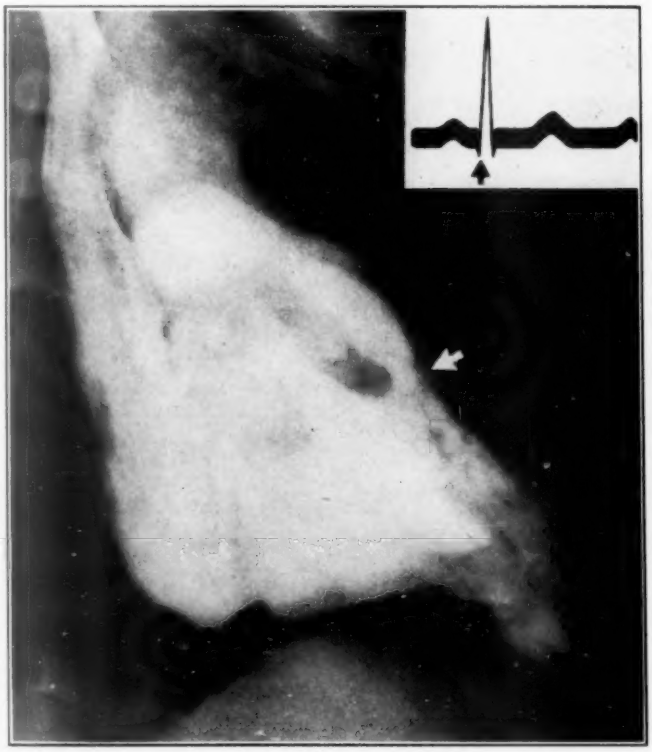

Fig. 7.—Tetralogy of Fallot, right anterior oblique projection near the end of atrial systole. The infundibulum of the right ventricle is much narrower than normal, particularly at its ostium (arrow).

The occurrence of stenosis in pulmonary arterial branches is attracting some interest at the moment. Multiple stenoses in peripheral branches have been described as a cause of right ventricular hypertension and failure (Arvidsson, Karnell and Möller[3]). More often, as in the case shown in Fig. 8, the stenosis is localized to the origin of each main branch in association with pulmonary valvular stenosis, ventricular septal defect or tetralogy of Fallot (Kjellberg *et al.*[26] and Stern, Figley and Talner[47]). In severe cases, as this one, the right ventricular output is impeded by the stenoses and collected temporarily between them and the pulmonary valve with resulting remarkably wide pulsation of the pulmonary artery trunk.

The clinical problem of distin-

guishing between mitral stenosis and insufficiency has been studied by angiocardiography. Zinsser's early conclusion that a distinguishing opacification pattern of the left heart chambers existed has been abundantly denied (Zinsser and Johnson[51], Steiner and Goodwin[46], Soloff *et al.*[44], and McAfee, Hilbish and Stewart[34]). Nevertheless

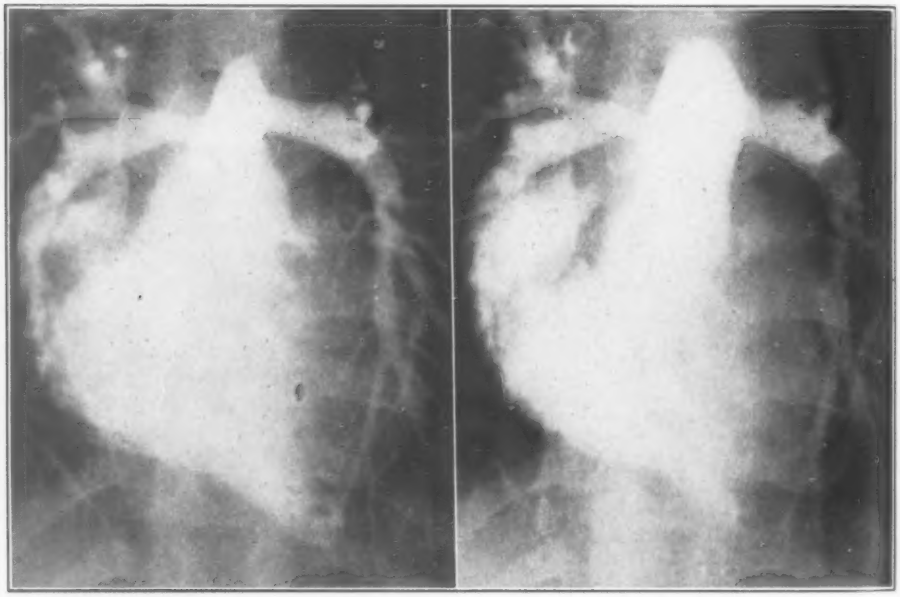

Fig. 8.—Pulmonary arterial stenosis, frontal projection. Associated pulmonary valvular stenosis is not visible in these reproductions. *Left*, ventricular diastole; *right*, ventricular systole. Note the large caliber change in the pulmonary arterial trunk and relatively slight change in the main branches . At catheterization, pressure gradients across each main branch orifice were shown.

the hemodynamic dissimilarities shown by left atrial pressure curves and electrokymography (Andersson[2]) can be supported angiocardiographically. In patients with normal sinus rhythm the relative variations in the volume of the left atrium have been determined by measuring the area of the atrial projections and plotting these against the ECG in patients with surgically verified dominant mitral stenosis or insufficiency (Judge, Figley and Sloan[25], and Arvidsson and Odman[4]). The resulting curves (Fig. 10) show that atrial emptying is impaired in mitral stenosis, occurring slowly during early ventricular diastole when it is normally rapid and to a relatively larger extent than normal during atrial contraction. On the other hand, in mitral insufficiency the atrium empties rapidly in early ventricular diastole and relatively very little during atrial systole. On the basis of these findings, electrokymographic records have been re-evaluated and found to show with few exceptions the principal valve lesions in a large group of surgical patients (Judge, Figley and Sloan[28]).

The Movement of Blood. Abnormalities may be encountered in the move-

ment of blood, both as regards its velocity and direction of flow, the latter being of considerably more practical importance.

Under the usual conditions at venous angiocardiography, the injected contrast material moves sufficiently rapidly down the superior vena cava that it appears to have mixed completely with nonopaque blood. Furthermore, the turbulence in the right atrium and ventricle is such that normally these chambers appear rapidly and uniformly opacified.

When, however, the heart chambers are dilated and the stroke volume of the ventricles is low, the movement of blood is sufficiently slow that defects in mixing of the contrast medium can be shown by the relatively long exposures of 1/10 to 1/20 second duration. These may be seen in the right atrium in heart failure, tricuspid stenosis, constrictive pericarditis, and other conditions. With severe pulmonary hypertension they may even be seen in the pulmonary artery (Fig. 11). When the contrast material can be seen to sediment in this vessel, as in this instance, the movement of blood is necessarily slower than normal.

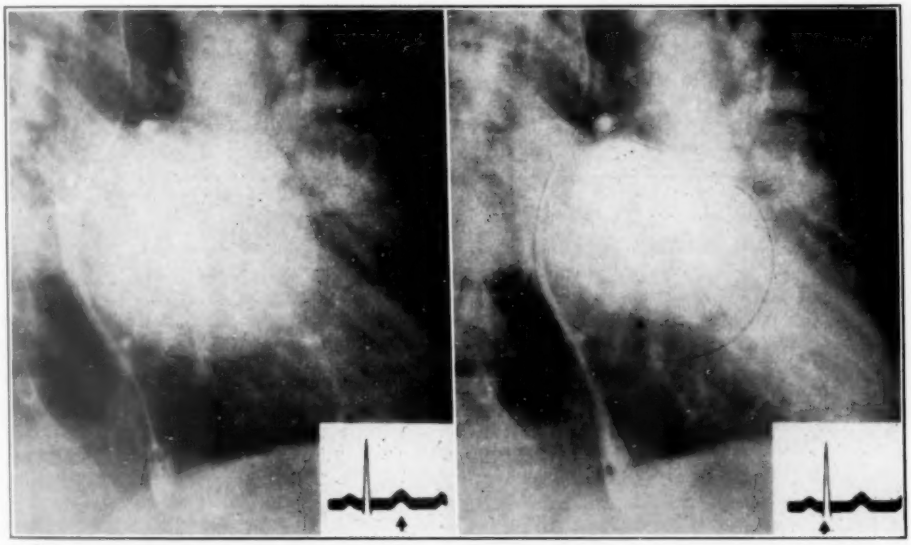

Fig. 9.—Mitral stenosis, right anterior oblique projection. *Left*, full distention of the left atrium near the end of ventricular systole. *Right*, near the end of atrial contraction. The size and position of the atrium on the left film have been penciled onto the right film. Note the wide amplitude movement of the A.V. plane and atrial floor compared to the slight movement of the barium filled esophagus against the posterior wall of the atrium. The curve "R.W." in Fig. 10 shows the relative area variations of this atrium.

Dotter[15] has suggested the possibility of calculating blood velocity using this principle by employing particulate contrast material and measuring its movement in a given period.

Efforts have been made to express the velocity of the circulation in terms of time taken for blood to move successively through the heart chambers (Figley[17], Nordenström[36], and Soloff *et al.*[44]). Some fairly broad ranges of

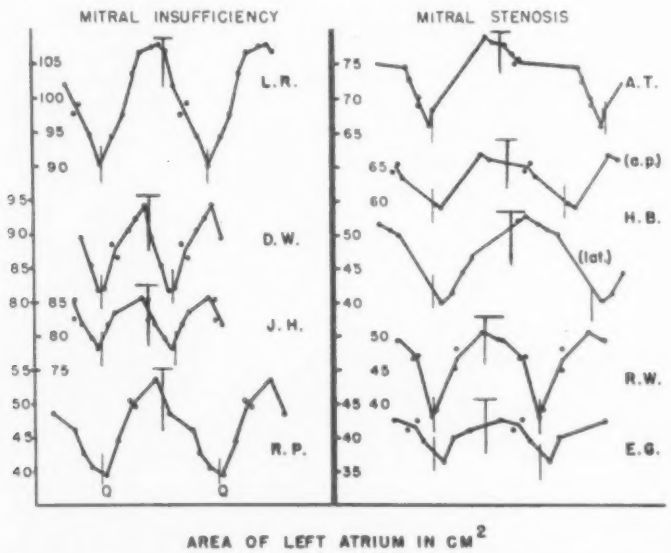

Fig. 10.—The cyclic variations in the areas of left atrial projections in 8 cases of surgically verified mitral disease, 4 tight stenosis, 4 dominant insufficiency. In one case, "H.B.," both frontal and lateral projections were available and showed similar cyclic variations indicating that volume change could be inferred from the area curves. Vertical lines mark position of the Q wave; crossed lines mark the end of the T wave. The manner of left atrial emptying following maximum atrial distention distinguishes the valvular abnormalities (see text).

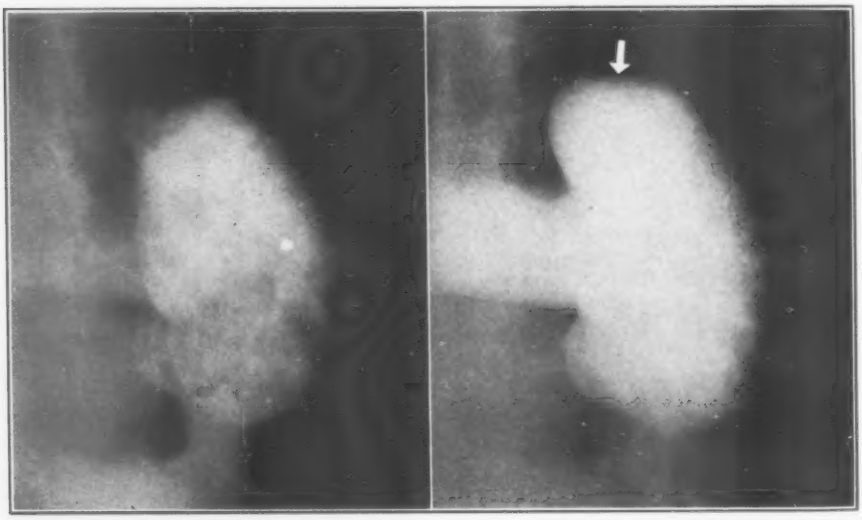

Fig. 11.—Pulmonary artery, frontal projection, in a patient with mitral stenosis and severe pulmonary hypertension. *Left*, beginning opacification of the artery. Mixing defects such as those seen here are not shown with the same filming technique in normal patients. *Right*, the artery, seen later, is fully opacified except at the superior margin of the left branch (arrow) where the contrast has sedimented.

normal have been given but without reference to pulse rate or respiration in the clinical material. It is to be expected that with more attention to these details more precise definition of the normal will be possible. Yet before these are available it may be shown that slow flow does not necessarily depend upon chamber dilatation. The right heart chambers are relatively normal in size in constrictive pericarditis where there seems to be definite delay in the circulation (Figley and Bagshaw[19]) (Fig. 12). The relation between heart volume and stroke volume would appear to be critical as a general principle in alterations of circulation velocity and offers an interesting problem for future angiocardiographic investigation.

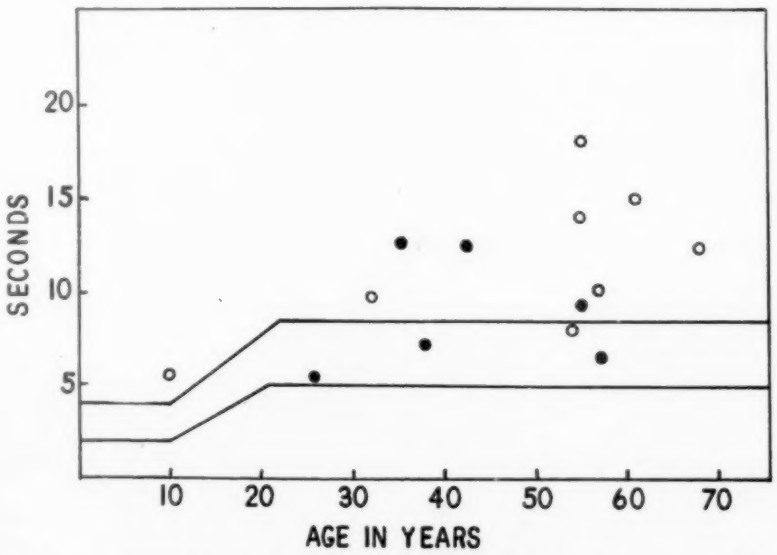

Fig. 12.—Angiocardiographic circulation time between initial superior vena caval and aortic opacification. Lines indicate upper and lower limits of normal. Open circles are cases of surgically verified constrictive pericarditis; closed circles are cases of pericarditis effusion. The two cases of the latter with the most delayed circulation had clinical signs of cardiac tamponade.

Abnormalities in direction of flow are susceptible to more precise recognition by angiocardiography. Right to left shunts are signaled by premature aortic opacification. In appropriate projection the location of these shunts, atrial, ventricular or arterial, can generally be determined by simple venous angiocardiography, although of course anatomic details are better shown by the selective method.

Left to right shunts, on the other hand, have in common recirculation of the contrast material through the pulmonary artery. The shunts may be localized however, by defects in the uniformity of the initial opacification of the right heart chambers (Kjellberg *et al.*[26], Lind, Spencer and Wegelius[31], and Figley *et al.*[20]). Such a shunt through a patent ductus quite regularly produces a mixing defect in the pulmonary artery that is most conspicuous in diastole (Figley *et al.*[20], and Campeti[6]). Furthermore, the bidirectional nature of this shunt, R→L in

ventricular systole and L→R in ventricular diastole, can be shown when the pulmonary artery and aortic pressures are equilibrated (Kjellberg *et al.*[26], and Figley *et al.*[20]). Ventricular septal defect with L→R shunts can similarly be localized in many cases by mixing defects in the right ventricle which are generally systolic in time (Fig. 13). The recognition of atrial

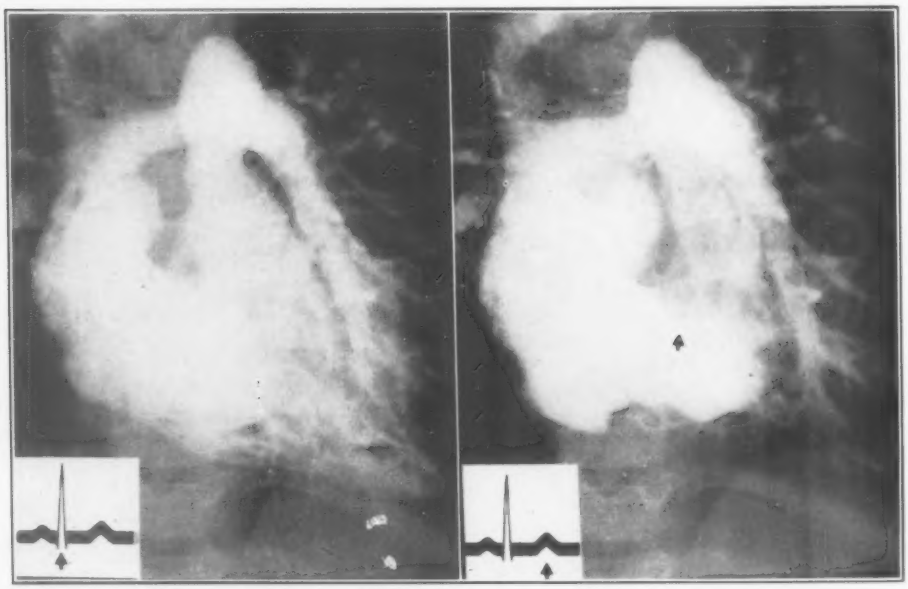

Fig. 13.—Ventricular septal defect with left to right shunt, frontal projection. *Left*, ventricular diastole near the end of atrial contraction. *Right*, near the end of ventricular systole. Note the dilution of contrast material in the right ventricular infundibulum above the arrow localizing the shunt to the ventricular level.

L→R shunts on this basis seems unreliable, however, due to the normal mixing defects in the right atrium from the venous return of unopacified blood from the other vena cava.

DISORDERED FUNCTION OF HEART VALVES. Stenosis of the tricuspid and pulmonary valves is probably most precisely established by right heart catheterization. The morphologic deformity of valvular pulmonic stenosis can be shown in the majority of cases by venous angiocardiography, but visualization is distinctly superior by injection of the contrast material into the right ventricle (selective angiocardiography [Jönsson, Brodén and Karnell[24], and Kjellberg *et al.*[26]]). Nevertheless a curious feature has been found in several cases by the venous method, namely elevation of the pulmonary valve during atrial systole (Fig. 14). This implies movement of the valve and a small amount of blood during ventricular diastole. To occur, the "a" wave of right atrial pressure must be higher than the pulmonary pressure at that instant. Such has been true in those cases where this movement has been shown (Figley and Rudhe[18]).

Stenosis of the mitral and aortic valves is not adequately shown by venous angiocardiography and some

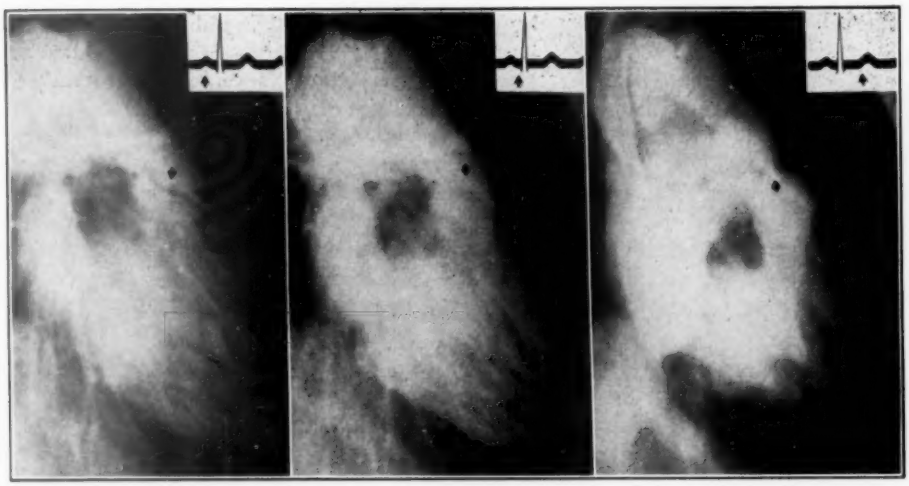

Fig. 14.—Pulmonary valvular stenosis, right anterior oblique projection. *Left*, near the onset of atrial contraction, the pulmonary valve is inverted (arrow); *center*, during atrial contraction the valve is elevated (arrow). *Right*, during ventricular ejection the opening in the valve (arrow) and jet are shown.

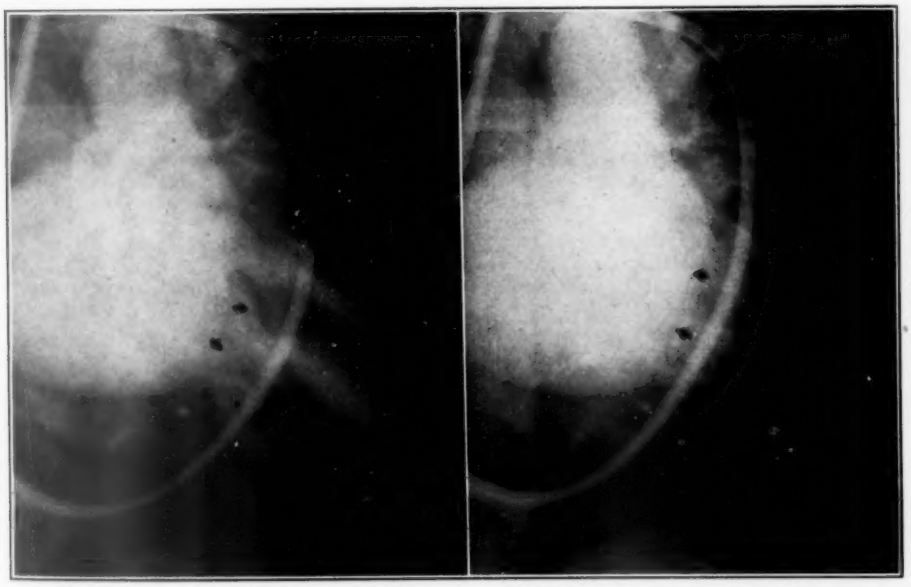

Fig. 15.—Mitral stenosis, right anterior oblique projection. Injection made by catheter in the pulmonary artery. *Left* during ventricular diastole the atrial base is dome-shaped and points toward the apex. The thickened anterior leaflet of the mitral valve is seen (upper arrow) and the opening in the valve (between arrows). *Right*, during ventricular systole the leaflets are thrust upward into the atrium.

method of selective opacification of the left heart chambers is required, such as injection by catheter into the pulmonary artery (Fig. 15), or by needle inserted into the left atrium (Rudhe[39]) or left ventricle (Lehman, Musser and Lykens[30]) (Fig. 16). The value of these methods is now being explored, for the assessment of combined valvular lesions has not been altogether satisfactory by clinical methods or left heart catheterization.

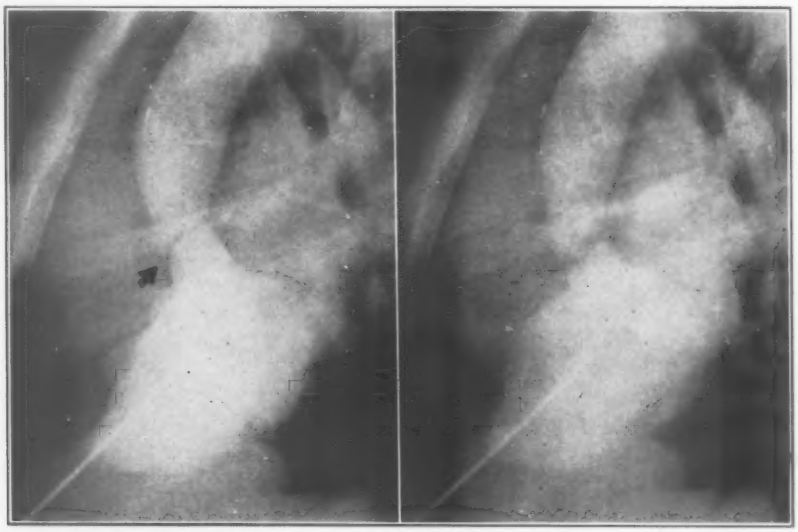

Fig. 16.—Congenital aortic stenosis and mitral incompetence. Female 12, lateral projection. Contrast material injected by needle placed percutaneously in the left ventricle. *Left*, at the end of injection during left ventricular ejection the stenosed aortic valve is shown (arrow). *Right*, three heart beats later, contrast material is also found in the left atrium, indicating mitral incompetence. This is regarded as slight and due to the left ventricular dilatation rather than organic valvular disease as it was not suspected clinically.

The recognition of valvular incompetency is also of pressing importance, particularly of the mitral valve. In principle, simple venous angiocardiography is unsuited for the direct demonstration of valvular incompetence. Selective introduction of the contrast material into the chamber distal to the valve is necessary for the retrograde flow through the valve to be clearly seen. It is not ideal to do this with a catheter through the valve in question in view of possible interference with its function. Needle puncture of the right ventricle is used for the demonstration of tricuspid insufficiency (Lehman, Musser and Lykens[30]), of the left ventricle for mitral insufficiency (Nordenström *et al.*[37], Lehman, Musser and Lykens[30], Smith, Gregg and Klassen[43], and Wilder, Moscovitz and Ravitch[50]) (Fig. 16), and by thoracic aortography for aortic insufficiency (Kjellberg *et al.*[26], and Wilder, Moscovitch and Ravitch[50]). Although incompetence of the normal mitral valve was not observed by Wilder[50] or Nordenström[37] and their associates, cineangiocardiographic studies by Stauffer and Oppenheimer[45] have clearly shown that the normal mitral valve will allow retrograde flow dur-

ing bradycardia. Since arrhythmia and ineffective contraction are almost constant features of present-day injections into the left ventricle, evaluation of the significance of mitral valve reflux must be made with due regard to the heart action at the time of filming.

Conclusion. The utimate goal of cardiology is the accurate recognition and correction of physiologic abnormalities. Angiocardiography makes a contribution to this goal today in relatively few cases and until recently has been devoted primarily to better appreciation of underlying anatomic deformity. But it is possible even with simple venous injection and filming equipment to show alterations in chamber contraction, in the flow of blood, and in valve action in certain circumstances if some timing device such as simultaneous electrocardiography is used. To date we have had only glimpses of such alterations in heart physiology. In the future with more elaborate equipment and application angiocardiography may well become a widely used method of physiologic investigation.

REFERENCES

1. **Amundsen, A. K., Amundsen, P.,** and **Miller, P.:** Acta radiol., **45,** 452, 1956.
2. **Andersson, T.:** Ibid., Suppl. 106, 1953.
3. **Arvidsson, H., Karnell, J.,** and **Möller, T.:** Ibid., **44,** 209, 1955.
4. **Arvidsson, H.,** and **Odman, P.:** Ibid., **47,** 97, 1957.
5. **Braunwald, E., Fishman, A. P.,** and **Cournand A.:** Circulation Res., **4,** 100, 1956.
6. **Campeti, F. L.:** Patent Ductus Arteriosus: Forty-one Cases Studied by Cineangiocardiography. Read at 28th Scientific Session, Am. Heart Assn., New Orleans, 22–24 October 1955.
7. Idem: Infundibular Dynamics Studied by Cineangoicardiography in Pure Valvular Pulmonary Stenosis. Read at 29th Scientific Session, Am. Heart Assn., Cincinnati, 27 October 1956.
8. **Chamberlain, W. E.:** Radiology, **38,** 383, 1942.
9. **Chamberlain, W. E.,** and **Dock, W.:** Ibid., **7,** 185, 1926.
10. Iidem: A.M.A. Arch. Int. Med., **40,** 521, 1927.
11. **Chamberlain, W. E.,** and **Henny, G. C.:** Am. J. Roentgenol., in press.
12. **Chamberlain, W. E., Stauffer, H. M.,** and **Bladestone, A. W.:** Scientific Exhibit, Am. Roentgen-ray Soc., 52nd Meeting, Washington, 1951.
13. **Coblentz, B., Harvey, R. M., Ferrer, M. I., Cournand, A.,** and **Richards, D. W.:** Brit. Heart J., **11,** 1, 1949.
14. **Dodge, H. T.,** and **Tanenbaum, H. L.:** Circulation, **14,** 927, 1956.
15. **Dotter, C. T.:** Ibid., **12,** 1034, 1955.
16. **Figley, M. M.::** Presented at 2nd Annual Meet. Assn. University Radiologists, St. Louis, 1954.
17. Idem: Radiology, **63,** 837, 1954.
18. **Figley, M. M.,** and **Rudhe, W.:** Unpublished observations.
19. **Figley, M. M.,** and **Bagshaw, M. A.:** Radiology, to be published.
20. **Figley, M. M., Nordenström, B., Stern, A. M.,** and **Sloan, H. E.:** Acta radiol., **45,** 425, 1956.
21. **Hedman, C., Lind, J.,** and **Wegelius, C.:** Brit. Heart J., **15,** 143, 1953.
22. **Henny, G. C.,** and **Boone, B. R.:** Am. J. Roentgenol. & Rad. Ther., **54,** 217, 1945.
23. **Henny, G. C., Boone, B. R.,** and **Chamberlain, W. E.:** Am. J. Roentgenol. & Rad. Ther., **57,** 409, 1947.
24. **Jönsson, G., Brodén, B.,** and **Karnell, J.:** Acta radiol., **40,** 547, 1953.
25. **Judge, R. E., Figley, M. M.,** and **Sloan, H. E.:** Circulation, in press.
26. **Kjellberg, S. R., Mannheimer, E., Rudhe, U.,** and **Jönsson, B.:** Diagnosis of Congenital Heart Disease. Chicago: The Year Book Publishers, Inc., 1955.
27. **Kjellberg, S. R.,** and **Olsson, S.:** Acta radiol., **41,** 481, 1954.

28. **Lagerlöf, H.,** and **Werkö, L.:** Cardiologia, **13,** 241, 1948.
29. **Leatham, A.:** Lancet, **2,** 607, 1954.
30. **Lehman, J. S., Musser, B. G.,** and **Lykens, H. D.:** Am. J. Roentgen. & Rad. Ther., **77,** 207, 1957.
31. **Lind, J., Spencer, R.,** and **Wegelius, C.:** Brit. Heart J., **16,** 407, 1954.
32. **Lind, J.,** and **Wegelius, C.:** Univ. of Michigan Med. Bull., **22,** 447, 1956.
33. **Lind, J., Wegelius, C.,** and **Lichtenstein, H.:** Circulation, **10,** 195, 1954.
34. **McAfee, J. G., Hilbish, T. F.,** and **Stewart, K. R.:** Radiology, **67,** 321, 1956.
35. **Nordenström, B.:** Acta radiol., **47,** 89, 1957.
36. Idem: Ibid., **41,** 209, 1954.
37. **Nordenström, B., Stamer, J. P., Figley, M. M.,** and **Sloan, H. E:** Circulation, **15,** 682, 1957.
38. **Rowe, G. W., Huston, J. H., Tuchman, H., Maxwell, G. M., Weinstein, A. B.,** and **Crumpton, C. W.:** Ibid., **13,** 896, 1956.
39. **Rudhe, W.:** Personal communication.
40. Idem: Acta radiol., Suppl. 134, 1956.
41. **Ruggles, H. F.:** Radiology, **5,** 444, 1925.
42. **Sawyer, C. G., Burwell, C. S., Dexter, L., Eppinger, E. C., Goodale, W. T., Gorlin, R., Harken, D. E.,** and **Haynes, F. W.:** Am. Heart J., **44,** 207, 1952.
43. **Smith, P. W., Gregg, H. A.,** and **Klassen, K. P.:** Circulation, **14,** 847, 1956.
44. **Soloff, L. A., Zatuchni, J., Stauffer, H. M.,** and **Kelly, E. W.:** Ibid., **13,** 334, 1956.
45. **Stauffer, H. M., Oppenheimer, M. J., Soloff, L. A.,** and **Steward, G. H.:** Am. J. Roentgenol. & Rad. Ther., **77,** 195, 1957.
46. **Steiner, R. E.,** and **Goodwin, J. F.:** J. Faculty of Radiologists, **5,** 164, 1954.
47. **Stern, A. M., Figley, M. M.,** and **Talner, N.:** To be published.
48. **Wegelius, C.,** and **Lind, J.:** Diagnostic Evaluation of Heart Dynamics by Angiocardiography. *In* Modern Trends in Diagnostic Radiology, 2nd Series, edited by **McLaren, J.** W. London: Butterworth & Co., Ltd., 1953.
49. **Wiggers, C. J.:** Physiology in Health and Disease. Philadelphia: Lea & Febiger, 1949.
50. **Wilder, R. J., Moscovitz, H. L.,** and **Ravitch, M. M.:** Surgery, **40,** 86, 1956.
51. **Zinsser, H. F., Jr.,** and **Johnson, J.:** Ann. Int. Med., **39,** 1200, 1953.

SUMMARIO IN INTERLINGUA

Aspectos De Physiologia Cardiac, Visualisate Per Medios Angiocardiographic

Le ultime objectivo del cardiologo es le accurate recognition e le subsequente correction de anormalitates physiologic del corde. In nostre dies le angiocardiographia contribue al realisation de iste objectivo in un relativemente micre numero de casos, e usque recentemente illo esseva primarimente dedicate a un meliorate comprension del subjacente deformitates anatomic. Nonobstante, il es possibile, mesmo per medio de simple injectiones venose e simple apparaturas angiocardiographic, demonstrar alterationes in le contraction del cameras, in le fluxo de sanguine, e in le action valvular sub certe conditiones si le un o le altere dispositivo chronometric (como per exemplo per electrocardiographia simultanee) es utilisate. Usque nunc nos ha obtenite non plus que vistas furtive de tal alterationes del physiologia cardiac. In le futuro, le uso de raffinate apparatos e methodos de application promitte facer del angiocardiographia un extensemente utilisate methodo de investigation physiologic.

USE OF A QUANTITATIVE ELECTROKYMOGRAPH IN EVALUATION OF MYOCARDIAL CONTRACTILITY

By Robert H. Eich, M.D.
ASSISTANT PROFESSOR OF MEDICINE

Yale Citrin, M.D.
INSTRUCTOR IN MEDICINE

Arthur Schreier, M.D.
FORMERLY RESIDENT IN MEDICINE

AND

Richard H. Lyons, M.D.
PROFESSOR AND HEAD, DEPARTMENT OF MEDICINE

WITH THE TECHNICAL ASSISTANCE OF

Paul Gabel, B.S.

(From the Department of Medicine, State University of New York College of Medicine, Syracuse, and the Veterans Administration Hospital, Syracuse, New York)

Early work with the electrokymograph (EKY) was in part stimulated by the hope that it would prove a direct measure of myocardial function. With increasing experience with the qualitative EKY, it soon became apparent that the motion of the left ventricle was too complex for a direct measure of myocardial contractility to be attained. As a result, in this country the EKY has become a very limited tool, used for timing cardiac events, and for studying left atrial changes. The present study represents a further refinement in calibration of the apparatus and its application to the study of myocardial contractility.

A method of calibrating the electrokymograph was first suggested by Henny in 1950[5]. Basically he accomplished this by simulating the addition of a known amount of heart border to the recording field. In front of the pickup unit he mounted a lead diaphragm which moved into the field, obscuring a known proportion of the total roentgenograms. This calibrated motion was added to the baseline of the electrokymogram.

Morgan and Sturm in 1951 described an electrokymograph which they felt could be quantitated in millimeters of border motion[9]. With this instrument, simulated border was added to the recording field by means of a sliding unit containing a Legow-Forchet Roentgen grid, a Patterson D screen and a diffusing lucite block. The unit was able to eliminate scatter Roentgen-ray and diffuse the resultant fluoroscopic light evenly over the exposed sensitive grid of the photoelectric tube, which remained fixed. The entire unit could be moved a known distance, for example, 5 mm. An improved amplifier circuit was also utilized in this electrokymograph.

The calibration method of Morgan and Sturm is theoretically sound. It requires, however, that the light sensitive grid of the RCA-931A photomultiplier tube be linear over a defini-

tive distance. The addition of a known amount of border density, to a new portion of the light sensitive grid, still must result in an equivalent linear response. Unless this can be demonstrated to be true for each tube, a quantitative record is not obtained. This system of calibration also requires that the Roentgen-rays striking the fluorescent screen be approximately parallel, so that an equivalent image is projected before and during calibration. The Roentgen grid is used to eliminate scatter radiation from falling upon the screen within the recording head. The equivalence of the border motion complexes before and during calibration is dependent in large part upon the effectiveness of this grid in eliminating the divergent radiation.

Any method of quantitating the electrokymograph makes it necessary that criteria be set up which define the limits for accuracy in calibration of each record. We have attempted a solution of this problem by constructing an artificial metallic border which can be moved a known distance. The accuracy of the quantitative electrokymograph can then be gauged by calculating this already known motion. Utilizing the quantitative electrokymograph as described by Morgan and Sturm[9], we have been unable to calculate consistently the motion of the artificial border. Certain modifications were found essential before we were successfully able to obtain accurate and reproducible calibration motion and comparable border motion complexes before and during calibration. Since the photomultiplier tubes are not uniformly linear along the length of their grids, the modifications of the electrokymograph were directed primarily toward the development of a method not requiring such linearity. It was, therefore, found necessary to move simultaneously the entire contents, including the photoelectric tube of the recording head, during the calibration. The same area of the photo-

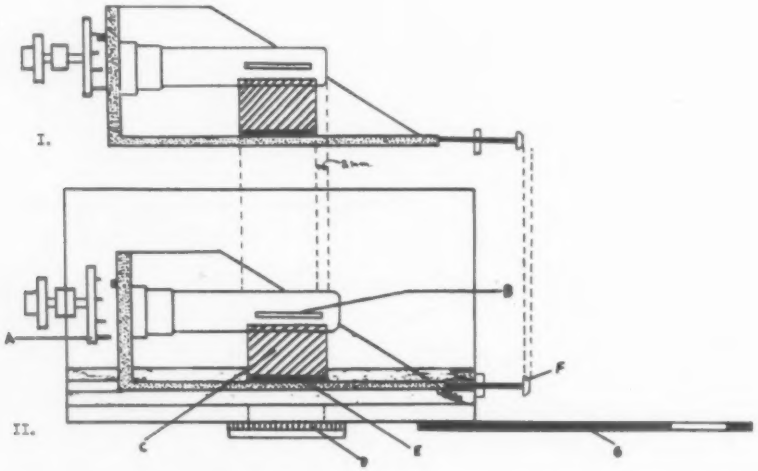

Fig. 1.—Cutaway view of EKY head. *A.* Calibrating stops. *B.* Light sensitive grid in P–931A tube (or 1–P21). *C.* Lucite block. *D.* 2 Schonander grids at 90° angles. *E.* Patterson D type fluoroscopic screen. *F.* Calibrating knob. *G.* Sighting arm.

multiplier tube is exposed throughout. Comparable complexes during all periods were not, however, obtained until divergent Roentgen-rays were more completely excluded by the substitution of two layers of Schonander grid, crossed exactly at 90 degree angles, in place of the conventional Roentgen grid. Although the intensity of the resultant signal was somewhat dampened, a more accurately calibrated record of motion was uniformly obtained. These changes have resulted in an electrokymograph, which we believe is reproducibly calibrated.

Material and Methods. A cross section of the modified recording head is illustrated in Fig. 1, positioned before and during calibration. The grids (*D*), after eliminating scatter Roentgen rays, allow polarized beams to pass through the aperture in the lead-lined, light-proof encasement box. Then, the Roentgen rays strike the Patterson D-type fluoroscopic screen (*E*). The emitted light enters the lucite block (*C*) which diffuses the light towards the light sensitive grid of the P–931A tube (*B*). Since the lucite block, tube, and screen all move as a unit, the same area of tube is always exposed.

The pickup unit is moved during calibration either manually by means of the calibrating knob (Fig. 1*F*) or, if desired, electrically by means of a solenoid. The movement is predetermined by setting into position one of the six calibrated stops (Fig. 1*A*), which range from 1 mm. through 6 mm.

The amplifying circuits are similar to those described by Morgan and Sturm[9], with essentially the same linear characteristics and frequency responses.

The electrokymographic unit is mounted on rigid horizontal tracks on the fluoroscopic screen of the Roentgen-ray table. The border motion is viewed through a comparable aperture in the end of a 30 cm. sighting arm (Fig. 1*G*). The electrokymographic head is then manually moved 30 cm. to the area sighted, and locked into place.

The accuracy of the EKY was assessed using the movement of the border of a metallic shield as a standard. The distance of the shield to the fluorescent screen and the amount of motion were both varied. The known border motion could be calculated from the electrokymogram with 10% error. The reproducibility was evaluated by comparing two tracings done at 20-minute intervals in 9 normal subjects. The left ventricular border motions of 22 young adults, with no evidence of cardiovascular disease, were then measured and compared with those of 9 patients with selected cardiovascular disease states. The latter group consisted of 2 patients with dynamically evident aortic insufficiency, one rheumatic, and one syphilitic in origin; 5 patients with compensated arteriosclerotic heart disease, associated with hypertension in 2; one patient with myocarditis; and one with proven constrictive pericarditis.

The records were obtained from two points along the border, the low and the mid-left ventricle. Lead II electrocardiogram, carotid pulse tracing and photocardiogram were recorded simultaneously with the electrokymogram. The Sanborn Poly-Viso recorder at a paper speed of 25 mm./sec. was used to record the four events. The usual carotid pulse recorder was modified by placing the pressure transducer close to the pickup unit to avoid appreciable time lag. The patients rested 10 minutes before the test was done. Tracings were taken in supine patients at full inspiration, as the most easily reproducible chest position. A decrease in border motion usually occurred in the first 5 to 10 beats. After this early change, the motion then became constant, and the tracing could be made. The necessity of breath holding for 10 to 20 seconds made this technique impractical in several dyspneic patients.

In addition to the above subjects, 6 normal students and 10 subjects with hypertensive or arteriosclerotic heart disease, or both, in borderline compensation were studied at rest and after a standard 30-step exercise, or if the latter was not achieved, after the point of moderately severe dyspnea was reached. Their electrokymograms were analyzed for the change in the velocity of contraction of the left ventricle provoked by mild exercise in the manner to be described below.

Results. Figure 2 shows a typical tracing from a normal subject. The height of the electrokymogram from its peak to its nadir is called the *recorded border motion*. The *known calibration motion* is the distance that the entire EKY unit is moved during calibration, and corresponds to the number of the calibration stop previously set. Two millimeters was usually used. The *recorded calibration motion* is the dis-

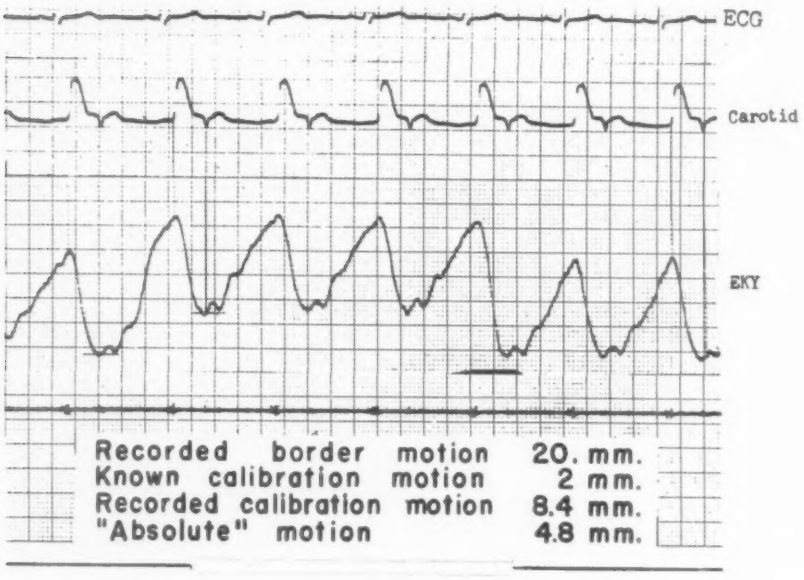

Fig. 2.—Typical tracing in a normal subject.

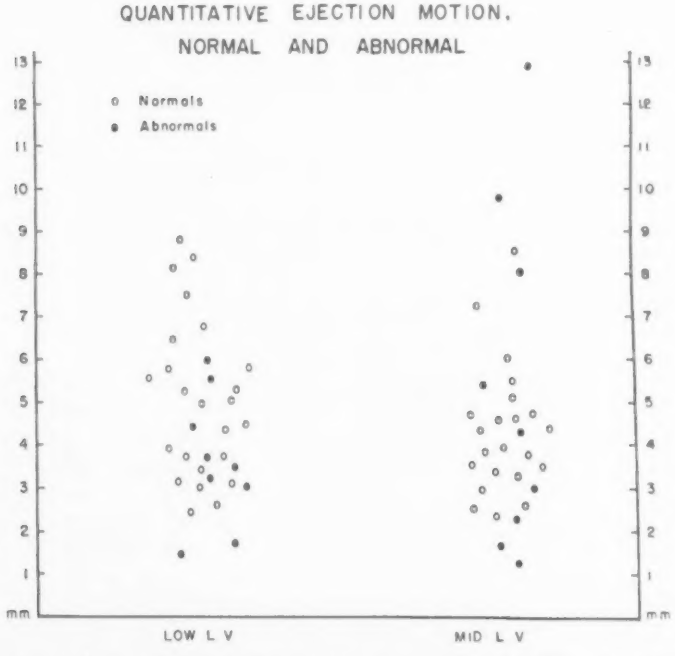

Fig. 3.—Left ventricular border motion in normal and abnormal subjects.

tance of the upward displacement of the baseline of the electrokymogram during the calibration maneuver. The *calibration factor* is obtained by dividing the *recorded calibration motion* by the *known calibration motion*. The *absolute motion* of the left ventricle is the *recorded border motion* divided by the *calibration factor*.

To obtain velocity, the slope of the mid-point of the ejection phase of the electrokymogram was extended for 1 sec. and expressed in millimeters per second. This slope divided by the *calibration factor* yields the *velocity index of systole* which, as will be discussed later, is felt to be a representation of the true maximum velocity of contraction.

Using the artificial border, it was found that the error was under 10% if the amplitude of the recorded border motion did not change more than 1 mm. before, during, or after calibration. Any record obtained that did not fulfill these criteria was discarded.

In Fig. 3, the amplitude of the motion of the border of the left ventricle is tabulated for both the normal and abnormal groups. There was no significant difference between the normal males and normal females. The motion did not correlate with body surface. Grouping the 22 normal male and female subjects together, the low left ventricular motion ranged from 2.4 to 8.9 mm. with a mean of 5.0 mm., standard deviation of 1.97. Motion along the mid-left ventricular border ranged from 2.4 to 8.4 with a mean of 4.3 and standard deviation of 1.47. There was no correlation in the amplitudes of the low-left ventricular motion with the mid-left ventricular motion. There was no significant difference between the normal and abnormal groups.

In Table 1 the maximum variation between serial determinations done 20 minutes apart in 9 subjects at complete rest are shown. The maximum variation obtained was 20%.

Table 2 shows the results of exercise on the various cardiac measurements in 6 normal subjects. All subjects showed a 2- to 3-fold increase in the velocity index, and a 1½- to 2½-fold increase in amplitude of border motion. While these two functions increased during exercise in a parallel fashion, systolic duration changed minimally, if at all.

TABLE 1.—REPRODUCIBILITY OF EKY TRACINGS AT REST

	20-Minute Intervals		
Case	*I*	*II*	*III*
1	2.4	2.0	2.0
2	5.8	5.2	
3	4.5	4.8	
4	6.7	6.9	
5	6.7	6.0	
6	3.0	2.9	3.0
7	3.2	3.1	
8	7.1	6.0	
9	5.1	4.5	

Table 3 shows the results of exercise on the cardiac measurements in 10 subjects with arteriosclerotic or hypertensive heart disease. These subjects had all been in frank heart failure in the past and were in varying degrees of complete compensation at the time of the study. Since most of these patients were in atrial fibrillation with extremely variable ventricular rates and rhythm, the ventricular rate and systolic duration were not felt to be amenable to ready analysis. These functions are, therefore, not included in Table 3. As shown the resting velocity index fell within the wide range of normal. In fact, the mean resting velocity index was 21 for both the normal and abnormal groups. However, there was a varying response to exercise in the patients with heart disease. In some, the

normal expected increase in velocity index did not occur. In fact, in several patients, the velocity of contraction decreased with exercise. Since no other index of the degree of compensation was assessed, it can not be stated categorically that those patients who did not show the expected increase in velocity were less completely compensated than those who did. It is, however, presumed that the degree of compensation was reflected in their response. The mean increase following exercise was 20% but it ranged from a decrease of 29% to an increase of 100%.

TABLE 2.—EFFECT OF EXERCISE ON THE LEFT VENTRICULAR BORDER MOTION IN NORMAL SUBJECTS

Subject	*Exercise*	*Cardiac Cycle sec.*	*Systol. sec*	*Absolute Border Motion mm.*	*Velocity Index*
1.	Control	1.0	0.28	1.0	9.6
	1 min.	0.96	0.28	2.0	14.0
	5 min.	0.88	0.29	2.7	16.8
2.	Control	0.72	0.28	3.5	22.2
	1 min.	0.64	0.24	5.3	45.2
	3 min.	0.64	0.24	4.6	63.0
3.	Control	0.88	0.28	3.7	26.0
	1 min.	0.80	0.32	8.2	76.0
4.	Control	0.72	0.24	2.9	22.0
	1 min.	0.76	0.24	6.0	55.0
	6 min.	0.75	0.24	3.6	32.8
5.	Control	0.96	0.30	2.8	15.0
	1 min.	0.92	0.30	4.6	30.0
	2 min.	1.04	0.32	3.9	21.0
	3 min.	1.10	0.32	5.0	26.0
	5 min.	0.96	0.32	3.3	21.6
6.	Control	0.86	0.30	7.7	30.4
	1 min.	0.78	0.26	10.1	83.2
	2 min.	0.78	0.26	13.3	113.2
	4 min.	0.80	0.27	10.4	88.0
	5 min.	0.81	0.29	8.4	53.4
	15 min.	0.82	0.30	8.2	47.0

Discussion. In the early work in electrokymography a striking similarity was noted between the left ventricular border motion, as recorded by the electrokymograph, and the volumetric curve of the ventricles as determined plethysmographically in dogs by Wiggers[14]. As has been pointed out by many investigators (Boone *et al.*[2,3], Lewis and Terry[7], and Morgan[8]), however, there is at best only a very general similarity between the two curves. The reasons for this discrepancy between the two measurements has been well appreciated by many authors (Akman *et al.*[1], Davies and Venning[4], Salans, Schack and Katz[11], and Schwedel Samet and Mednick[12]). There are certain nonvolumetric changes due to ventricular contraction superimposed on the border motion, particularly during the isometric contraction, early ejection and isometric relaxation phases. These motions, such as the pendulum motion of the entire heart, and rotational motions, will tend to be masked by the plethysmograph. The electrokymograph, however, records these changes, which are extremely variable both in extent and duration, depending both on the individual and

the site chosen along the left ventricle to record border motion. Because these nonvolumetric motions are superimposed on actual volumetric contraction of the ventricle, the resultant electrokymogram may show an extremely variable form in both normal and diseased hearts (Boone, Ellinger and Gillich[3]). In addition, differences in both size and shape of the heart affect the amplitude of border motion and cannot be adequately assessed at the present time.

TABLE 3.—EFFECT OF EXERCISE ON THE LEFT VENTRICULAR BORDER MOTION IN SUBJECTS WITH HEART DISEASE

Subject	*Exercise*	*Absolute Border Motion mm.*	*Velocity Index*
1.	Control	4	20
	2 min.	1.8	15
2.	Control	8.3	34
	2 min.	3.6	20
3.	Control	2.0	20
	2 min.	3.6	36
4.	Control	2.2	15
	2 min.	2.2	14
5.	Control	3.0	14
	2 min.	3.0	15
6.	Control	4.0	23
	2 min.	2.0	24
7.	Control	3.8	30
	2 min.	2.5	26
8.	Control	2.8	7.1
	2 min.	2.9	14.5
9.	Control	1.5	15.4
	2 min.	2.0	20
10.	Control	4.7	27.7
	2 min.	2.3	40.5

Although the noncalibrated EKY has not proved of great value in the assessment of the physiological state of the myocardium, it was hoped that a calibrated machine would extend its range of usefulness. With a calibrated machine, correction is made for changes in amplitude due to variations in Roentgen ray and gain from person to person. Our modification of the basic apparatus of Morgan and Sturm[9] when checked by an artificial border appeared to be calibrated. Its usefulness on a clinical level was then evaluated. It soon became apparent that many of the limitations of the noncalibrated EKY were still present. Only the ejection phase of systole with its well-established time limits could be subjected to quantitative analysis. Measuring the border motion of 22 normal subjects and 9 subjects with heart disease, no significant difference would be found between the two groups. In retrospect, the reason for this is apparent. Nonvolumetric positional changes, which are not directly related to ventricular functions, are recorded early and late in ejection (Morgan[8]) by the EKY whether it be quantitative or not. Further, the absolute motion of the border of the left ventricle bears little relation to function if assessed independently of heart size. A large heart can have the same stroke volume as a small heart with a much smaller systolic motion of the ventricular wall. However, variations in the functional state of the myocardium should be measurable in terms of changes in myocardial "contractility." The term "contractility" includes both the extent and the rate of myocardial shortening (Rushmer[10]). Wiggers[13] in his work on ventricular volume showed that while ejection did not occur at a constant velocity, the mid-portion of ejection was free of positional changes, and that changes in velocity play a major role in altering systolic discharge. With the qualitative EKY, it was observed that the steeper the slope of the ejection phase, the more rapid the cardiac action (Morgan[8]). Similarly, Kjellberg with a noncalibrated EKY confirmed the increased speed of contraction expected following epinephrine administration[6]. We, therefore, used the slope of the mid-portion of ejection as an index of velocity of contraction and

thus presumably of myocardial contractility.

The same limitations, due to heart size, shape, and muscle arrangement, would still hold for the resting velocity. Therefore, it is not unexpected that the resting velocity of the abnormals fall into the wide range of resting normal values.

When the change in velocity of myocardial contraction in response to exercise is utilized, however, the factors discussed above which limit the usefulness of the EKY probably tend to become less important. The expected pronounced increase in the velocity of contraction with exercise was found in the normal group.

The response to exercise was distinctly different from the normal in most subjects in borderline cardiac compensation. Thus, the 6 normals had a mean increase in velocity index of 162% with a range of 75 to 270% increase. The 10 abnormals had a mean increase of 20% with a range of 29% decrease to 100% increase. Obviously, more cases in both groups are needed, but it is suggested that the study of the velocity of contraction may prove a useful measure of not only the degree of compensation of the failing heart but also of the effect of various drugs and exercise on one aspect of myocardial function.

Summary. A modification of the calibrated electrokymograph designed by Morgan and Sturm is described. It is believed that accurately calibrated records can be obtained with this instrument.

A preliminary study of the motion of the border of the left ventricle is presented. An almost threefold variation in amplitude of border motion was observed among the normal subjects studied. The determinations in patients with heart disease fell within the normal range.

Despite calibration, the actual border motion of the left ventricle has little meaning until similar quantitation of the chamber size can simultaneously be made in man. The calibration, however, permits the introduction of a "velocity index" which may reflect the true maximum velocity of the contraction of the myocardium. Normal subjects showed the expected marked increase in velocity in response to exercise. This was not true, however, in most patients with arteriosclerotic or hypertensive heart disease, or both, in borderline cardiac compensation.

Although the results of this preliminary study are not statistically significant, they suggest that the calibrated electrokymograph may be a means of measuring myocardial contractility *in vivo*.

REFERENCES

1. **Akman, L. C., Miller, A. J., Silber, E. N., Schack, J. A.,** and **Katz, L. N.:** Circulation, **2**, 890, 1950.
2. **Boone, B. R., Chamberlain, W. E., Gillich, F. G., Henny, G. C.,** and **Oppenheimer, M. J.:** Am. Heart J., **34**, 560, 1947.
3. **Boone, B. R., Ellinger, G. F.,** and **Gillich, F. G.:** Ann. Int. Med., **31**, 1031, 1949.
4. **Davies, L. G.,** and **Venning, D. R.:** Brit. Heart J., **14**, 33, 1952.
5. **Henny, G. C.:** Electrokymography. *In* Medical Physics, edited by **J. Glasser.** Chicago: Year Book Publishers. Inc. Pp. 924–929, 1950.
6. **Kjellberg, S. R., Rudle, U.,** and **Sjostrand, T.:** Acta physiol. Scandinav., **24**, 333, 1952.
7. **Lewis, D. L.,** and **Terry, L. L.:** Ann. Int. Med., **32**, 36, 1950.
8. **Morgan, R. H.:** Am. J. Med. Sci., **218**, 587, 1949.
9. **Morgan, R. H.,** and **Sturm, R. E.:** Circulation, **4**, 604, 1951.
10. **Rushmer, R. F.:** Physiol. Rev., **36**, 400, 1956.

11. **Salans, A. H., Schack, J. A.,** and **Katz, L. N.:** Circulation, **2,** 900, 1950.
12. **Schwedel, J. B., Samet, P.,** and **Mednick, H.:** Proc. Soc. Exper. Biol. & Med., **73,** 591, 1950.
13. **Wiggers, C. J.:** J. Pharm. & Exper. Therap., **30,** 217, 1926–27.
14. Idem: Circulation in Health and Disease. Philadelphia: Lea & Febiger, 1923.

SUMMARIO IN INTERLINGUA

Le Uso de Un Electrokymographo Quantitative in le Evalutation del Contractilitate Myocardial

Es describite un modification del calibrate electrokymographo de Morgan e Sturm. Es asserite que registrationes de calibration accurate pote esser obtenite per medio de iste instrumento.

Un studio preliminar del motion del margine del ventriculo sinistre es presentate. Un quasi triple variabilitate del amplitude del motion del margine esseva observate inter le subjectos normal qui esseva studiate. Le determinationes in patientes con insufficientia cardiac monstrava valores intra le limited del norma.

In despecto de calibration, le ver motion del margine del ventriculo sinistre va remaner sin grande signification usque il deveni possibile facer simultaneemente un simile quantitation del dimensiones del camera in humanos. Tamen, le calibration permitte le introduction de un "indice de velocitate" que reflecte forsan le ver maximo de velocitate del contraction in le myocardio. Subjectos normal monstrava le expectate marcate augmento del velocitate in responsa a exercitio. Sed isto non valeva in le majoritate del patientes con morbo cardiac arteriosclerotic o hypertensive o ambes in compensation cardiac marginal.

Ben que le resultatos de iste studio preliminar non es statisticamente significative, illos suggere que le electrokymographo calibrate es possibilemente un medio pro mesurar le contractilitate myocardial in vivo.

LABORATORY AND CLINICAL OBSERVATIONS ON THREE SIMILAR DIURETIC AGENTS: AMINOPHYLLINE, AMINOMETRADINE (MICTINE) AND AMINOISOMETRADINE (ROLICTON)

By Ralph V. Ford, M.D.
ASSISTANT CLINICAL PROFESSOR OF MEDICINE AND PHARMACOLOOGY
BAYLOR UNIVERSITY COLLEGE OF MEDICINE

John H. Moyer, M.D.*
ASSOCIATE PROFESSOR OF PHARMACOLOGY
BAYLOR UNIVERSITY COLLEGE OF MEDICINE

Carroll A. Handley, Ph.D.
CHAIRMAN AND PROFESSOR OF PHARMACOLOGY
BAYLOR UNIVERSITY COLLEGE OF MEDICINE

Charles L. Spurr, M.D.†
ASSISTANT DIRECTOR OF PROFESSIONAL SERVICES FOR RESEARCH, VETERANS ADMINISTRATION HOSPITAL; ASSOCIATE PROFESSOR OF MEDICINE, BAYLOR UNIVERSITY COLLEGE OF MEDICINE

AND

J. B. Rochelle, III, M.D.
SENIOR RESIDENT, INTERNAL MEDICINE, VETERANS ADMINISTRATION HOSPITAL

(From the Departments of Medicine and Pharmacology, Baylor University College of Medicine and the Veterans Administration Hospital, Houston, Texas)

The currently expanding field of diuretic therapy has been greatest in the development of oral diuretics. These compounds have different chemical structures and as a result variable modes of action; organomercurials, carbonic anhydrase inhibitors, acidifying salts, xanthines and pyrimadine derivatives, and others. These compounds frequently have similar and overlapping pharmacodynamic actions, as, for example, chlorothiazide, which has some properties common to both other groups (organomercurials and carbonic anhydrase inhibitors).

The compounds of the current report are members of the pyrimadine derivative group of diuretic agents (aminoisometradine‡, aminometradine§ and a xanthine, aminophylline, which have similar pharmacodynamic properties. This report consists of clinical and laboratory observations on renal hemodynamics and the excretion of water and various electrolytes following the administration of these three drugs. A determination of potency, based on their ability to increase sodium excretion was done. These results serve as the basis for comparison of these diu-

*Present address: Professor of Medicine, Hahnemann Medical College, Philadelphia, Pa.

†Present address: Professor of Medicine, Bowman Gray School of Medicine, Winston-Salem, N.C.

‡Rolicton (G. D. Searle & Co.).

§Mictine (G. D. Searle & Co.).

retic agents with each other and with other currently available ones. The rationale of this approach to the study of diuretic agents and results obtained in other drugs have been previously elaborated (Ford *et al.*[1], Ford, Moyer and Spurr[2,3,4] and Moyer *et al.*[7]).

Methods and Materials. LABORATORY OBSERVATIONS. Three groups of dogs were studied. The dogs in Group I received aminophylline. The dogs in Group II received aminoisometradine (Rolicton). Dogs in Group III received aminometradine (Mictine).

Group I. There were 15 dogs in Group I which received aminophylline intravenously. The doses employed were 5 mg./kg. in 5 dogs, 10 mg./kg. in 5 dogs, and 15 mg./kg. in 5 dogs. These dogs were not hydrated. The animals were taken from their cages on the morning of the study, anesthetized with 30 mg./kg. pentobarbital, and then catheterized. The control observations consisted of three 20-minute urine collection periods. The aminophylline was then given intravenously and successive 20-minute urine collection periods were obtained over a period of 3 hours (Handley, Chapman and Moyer[5]). Observations were made on urine volume and sodium and potassium excretion. Observations were not made on glomerular filtration rate following the administration of aminophylline since a previous study indicated that there was no significant alteration following the intravenous administration of a dose of 20 mg./kg.

Group II. There were 24 dogs in Group II which received aminoisometradine (Rolicton). Observations on renal hemodynamics were made on 16 dogs, employing doses of 5 mg./kg. and 20 mg./kg. given intravenously. Eight dogs were not hydrated and were studied in a manner similar to the animals in Group I which received aminophylline. The dose of aminoisometradine (Rolicton) employed in the 8 unhydrated dogs was 30 mg./kg. given intravenously. Creatinine clearance was used to measure glomerular filtration rate, and para-aminohippurate clearance was used to determine renal plasma flow. Renal blood flow was calculated from the renal plasma flow and hematocrit. Simultaneous observations were made of water and electrolyte excretion. Methods and techniques for this procedure have been described previously (Handley, Sigafoos and La Forge[6], and Moyer and Handley[8]).

Group III. There were 24 dogs in Group III which received aminometradine (Mictine). Eight of these were unhydrated and were studied in a manner similar to that employed on the dogs in Group I which received aminophylline. The dose of aminometradine (Mictine) was 30 mg./kg. given intravenously. Renal hemodynamic studies were done on the 16 hydrated dogs similar to the dogs in Group II which received aminoisometradine. Eight of these received 5 mg./kg. of aminometradine and 8 received 20 mg./kg. Simultaneous observations were made on water and electrolyte excretion during the renal function studies.

CLINICAL BIOASSAY STUDIES. The technique of this study has been described previously (Ford, Spurr and Moyer[3]). Briefly, 10 male patients with a roughly equivalent degree of well controlled congestive heart failure are the subjects of the study for each drug. These patients are maintained in an air-conditioned metabolic ward under the observation of trained personnel. With the exception of diuretic agents, all medications required for control of their heart failure are continued throughout the study. The patients are allowed to be ambulatory about the metabolic ward. The average diet contains approximately 300 gm. of carbohydrate, 100 gm. of protein, and 100 gm. of fat with a total of 2800 calories per day. This diet varies somewhat with the caloric requirements of the individual patient. The sodium content of each patient's diet is exactly 50 mEq. per 24 hours. The patients drink 3000 ml. of distilled water every day. They are allowed to equilibrate on this diet until each patient excretes a constant amount of sodium, which approximates 90% (45 mEq./24 hr.) of their dietary sodium. The diuretic agent to be tested is then administered. Before the administration of the next dose of a diuretic agent, several days (4 to 6) are allowed for the patient to regain his body weight by the restoration of sodium and water stores.

Each patient is weighed before breakfast, and after voiding. Urine is collected in 24-hour periods and analyzed for sodium, potassium, chloride, volume, and creatinine (as a test of the accuracy of the collection). Other routine studies include plasma electrolytes, electrocardiograms, and chest roentgenograms on each patient.

A complete electrolyte excretion pattern was obtained on patients receiving two of the three drugs (aminophylline and aminoisometradine). Each patient drank 3000 ml. of distilled water per day. This was allotted fractionally to assure a constant water intake throughout the day. Urine was collected under

oil in 6 periods per 24 hours (three 2-hour periods and three 6-hour periods). The specimens were analyzed for sodium, potassium, chloride, phosphate, titratable acidity, ammonia, bicarbonate, total solutes, and volume. Aminophylline was administered intravenously, 500 mg. over a 30-minute period. Aminoisometradine was administered orally as a single dose of 800 mg.

Dosage response relationships were determined on aminophylline, aminoisometradine (Rolicton), and aminometradine (Mictine). Observations on these drugs were then compared to currently available diuretic agents. Aminophylline (orally administered) has been previously studied (Ford *et al.*[1]) and showed a potency estimation of less than 0.5* which did not fullfill our previously established criteria for further study. This was especially true since even at the dose of 1200 mg./day, the toxicity was 25%. In view of the fact that aminoisometradine (Rolicton) and aminometradine (Mictine) were similar to aminophylline, yet relatively nontoxic, a more complete study was done on them. Aminoisometradine (Rolicton) was given orally in single doses, starting with a dose of 200 mg. and progressively increased (200, 400, 800, 1600, 2400 mg.) until it was seen that a dose of 2400 mg. produced nausea and vomiting in over 25% of the patients without any further augmentation of sodium excretion. Since doses of 800 to 1600 mg. produced optimum sodium response without excessive toxicity, the bioassay on 10 patients was limited to these two dosages. Aminometradine (Mictine) was given orally in single dosages of 150, 300, 600, 1200 and 1800 mg. until nausea and vomiting were observed at the 1800 mg. dose. There was no greater excretion of water or sodium at 1800 mg. than at 1200 mg. The optimum dosage response was found between 600 and 1200 mg., therefore, the bioassay was performed at these two dosages.

Results. LABORATORY OBSERVATIONS. *Group I.* Aminophylline in a dose of 5 mg./kg. produced a significant increase in both sodium and water excretion in unhydrated dogs (Table 1). Employing a dose of 10 and 15 mg./kg. enhanced the response observed at a dose of 5 mg./kg. (Table 1). The statistical significance was greater at the 10 mg./kg. dose due to the marked variability of response when the larger dose of 15 mg./kg. was employed.

Group II. When a 5 mg./kg. dose of aminoisometradine (Rolicton) was given there was no significant effect on blood pressure. However, at a dose of 20 mg./kg,. there was a slight reduction in blood pressure to 93% of the control value ($P<0.05$). This decrease in blood pressure was only temporary and returned to control values within one hour after drug administration. Glomerular filtration rate was not altered significantly (Table 2) with either dose of the drug. However, renal blood flow at first increased slightly after drug administration but within 2 hours it decreased to below the control values. The latter effect on renal blood flow is frequently observed following prolonged anesthesia and is probably unrelated to any response to the aminoisometradine.

The dose of 5 mg./kg. of aminoisometradine (Rolicton) did not appear to affect urine volume or sodium excretion significantly in the hydrated dogs on which renal function studies were done. However, at a dose of 20 mg./kg. there was a significant increase in both urine volume and sodium excretion ($P<0.01$), increasing urine volume from an average of 1.3 ml./min. for the group to a maximum of 3.2 ml./min. (Table 3*B*). The increase in sodium excretion was similar. There was a moderate increase in potassium excretion.

In the 8 unhydrated dogs (Table 4) there was a significant increase ($P<0.001$) in water excretion as well as in sodium ($P<0.01$) and potassium ($P<0.05$) excretion. This response persisted throughout the 3-hour period of

*Potency estimation of less than 0.5 indicates that aminophylline orally administered is less than one-half as potent as the standard (meralluride or Mercuhydrin) parenterally administered, that is, potency estimation of meralluride is one.

TABLE 1.—EFFECTS OF AMINOPHYLLINE ON WATER AND ELECTROLYTE EXCRETION

Dog No.	*Wt. kg.*	*Dose mg./kg.*	*Urine Volume ml./min.*						*Sodium μEq./min.*						*Potassium μEq./min.*					
			C	D_1	D_2	D_3	D_4	*Max.*	*C*	D_1	D_2	D_3	D_4	*Max.*	*C*	D_1	D_2	D_3	D_4	*Max.*
1	10.0	15.0	.17	1.43	1.38	.98	1.08	1.75	7	141	143	61	27	184	9	40	45	32	23	51
2	10.0	15.0	.08	.80	.20	.20	.23	.85	5	74	13	8	11	75	5	29	7	8	13	32
3	13.0	15.0	.20	.33	.43	.33	.23	.50	12	52	64	47	30	75	4	10	23	24	20	27
4	20.0	15.0	.18	.20	.18	.15	.23	.25	2	11	13	7	20	28	8	19	20	19	30	37
5	17.0	15.0	.08	.33	.58	.28	.24	.60	2	17	34	11	5	35	6	13	22	13	13	25
Average			.14	.62	.55	.39	.40	.79	6	59	53	27	19	79	6	22	23	19	20	34
P Value<				.20	.20	.20	.20	.10		.10	.20	.20	.05	.10		.05	.05	.05	.01	.01
1	16.0	10.0	.17	.48	.40	.30	.28	.45	18	91	84	75	45	96	5	24	29	44	38	45
2	15.0	10.0	.13	.55	.50	.35	.33	.60	14	44	28	17	12	46	7	9	9	8	4	9
3	14.0	10.0	.07	.45	.48	.60	.30	.60	9	132	179	200	53	225	3	27	37	35	19	42
4	16.0	10.0	.27	.50	.55	.40	.34	.60	36	161	218	136	96	223	14	22	20	16	16	26
5	15.5	10.0	.10	.25	.60	1.25	.45	1.30	8	14	64	175	44	195	1	11	26	49	25	52
Average			.15	.45	.51	.58	.34	.71	17	88	115	121	50	157	6	19	24	30	20	35
P Value<				.01	.01	.10	.05	.05		.05	.05	.05	.05	.05		.05	.05	.10	.20	.05
1	11.0	5.0	.22	.50	.55	.88	.83	1.05	2	64	77	125	103	125	6	8	10	11	13	17
2	12.0	5.0	.13	.43	.45	.40	.44	.60	19	77	114	86	88	148	4	3	13	18	27	45
3	13.0	5.0	.22	.58	.53	.53	.58	.80	2	69	56	68	87	103	11	27	22	14	15	35
4	10.0	5.0	.08	.20	.15	.20	.20	.30	10	25	24	32	23	32	10	15	11	14	13	18
5	11.0	5.0	.12	.17	.13	.12	.16	.30	5	6	3	5	5	10	5	6	5	7	9	14
Average			.15	.38	.36	.43	.44	.61	8	48	55	63	61	84	7	12	12	13	15	26
P Value<				.05	.05	.10	.05	.05		.05	.10	.10	.10	.05		.20	.10	.10	.20	.05

C = Control values: average of 3 twenty-minute urine collection periods.
D_1 = First 20-minute urine collection period after aminophylline.
D_2 = Second 20-minute urine collection period after aminophylline.
D_3 = One hour after drug administration (average of 2 twenty-minute urine collection periods).
D_4 = Two hours after drug administration (average of 2 twenty-minute urine collection periods).
Max. = Maximum response during any one 20-minute urine collection period.

TABLE 2.—THE EFFECT OF AMINOISOMETRADINE (ROLICTON) ON RENAL HEMODYNAMICS

		Mean Blood Pressure mm.Hg					*Glomerular Filtration Rate ml./min.*					*Hematocrit*					*Renal Blood Flow ml./min.*				
Dog No.	*Wt. Kg.*	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4
Group A									*5 mg./kg. Rolicton given intravenously*												
1	10.0	137	134	137	161	159	43	40	39	37	46	26	30	32	30	27	224	311	276	156	136
2	15.0	159	143	150	160	160	42	40	43	43	45	45	48	46	45	42	227	262	274	182	150
3	11.0	191	176	189	184	193	39	39	33	30	34	56	52	49	47	45	218	215	165	145	160
4	11.0	82	88	105	126	120	27	22	22	20	21	32	30	28	27	24	168	151	100	78	75
5	8.0	143	138	129	137	157	25	27	29	33	30	43	44	37	32	31	114	159	178	96	87
6	9.5	103	100	94	110	112	35	37	37	35	39	45	47	44	40	39	155	206	230	138	187
7	9.0	135	136	134	146	—	34	33	29	32	—	37	40	38	33	—	211	215	200	142	—
8	15.0	137	130	126	119	109	39	46	52	45	54	40	41	38	37	37	187	254	190	168	181
Mean	11.1	136	131	133	143	144	36	36	36	34	38	41	42	39	36	35	188	222	202	138	139
% of Control			96	98	105	106		100	100	94	106		102	95	88	85		118	107	73	74
P Value*<			.10	.50	.50	.40		.50	.50	.50	.20		.30	.20	.05	.05		.05	.50	.01	.20
Group B									*20 mg./kg. Rolicton given intravenously*												
1	10.0	128	111	120	132	148	30	28	27	30	29	29	32	26	25	25	145	165	128	117	103
2	15.0	106	99	106	107	113	53	49	43	46	49	42	48	45	37	43	333	362	240	190	219
3	10.0	133	136	138	136	125	40	49	49	42	46	40	41	45	46	45	225	308	298	191	196
4	14.0	131	118	136	147	162	35	29	26	31	37	47	35	32	29	30	219	177	115	101	117
5	9.5	108	98	106	104	119	42	45	41	44	47	30	32	30	26	34	246	219	167	150	185
6	15.0	93	98	99	110	116	30	35	28	28	27	41	40	40	38	42	227	208	135	103	110
7	14.0	135	124	119	130	135	32	21	20	21	19	32	34	33	32	28	135	138	109	103	78
8	9.0	138	131	145	148	152	38	37	37	37	38	36	38	40	38	39	203	269	235	165	143
Mean	12.1	122	114	121	127	134	38	37	34	35	37	37	38	36	34	36	217	231	178	140	144
% of Control			93	99	104	110		97	89	92	97		103	97	92	97		106	82	65	66
P Value*<			.05	.50	.20	.05		.50	.20	.10	.40		.50	.50	.30	.01		.40	.20	.01	.001

Key to abbreviations:

C = Control observations (average of 3 ten-minute urine collection periods)
D_1 = Observations immediately after administration of drug intravenously (3 ten-minute periods)
D_2 = Observations one hour after administration of drug (2 ten-minute periods)
D_3 = Observations two hours after administration of drug (2 ten-minute periods)
D_4 = Observations three hours after administration of drug (2 ten-minute periods)

$$* t = \bar{x} \sqrt{\frac{n(n-1)}{S_x 2}}$$

TABLE 3.—THE EFFECTS OF AMINOISOMETRADINE (ROLICTON) ON WATER AND ELECTROLYTE EXCRETION

	Urine Volume ml./min.					*Urine Sodium μEq./min.*					*Urine Potassium μEq./min.*					*Plasma Sodium mEq./L.*					*Plasma Potassium mEq./L.*				
Dog No.	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4
Group A											*5 mg./kg. Rolicton given intravenously*														
1	0.5	0.8	0.6	0.8	1.4	24	58	24	18	104	13	21	20	27	49	120	127	128	134	132	3.6	3.6	3.7	3.8	4.1
2	0.3	0.5	0.7	1.0	1.0	15	14	20	28	78	4	16	24	23	40	126	—	125	126	131	2.9	—	3.0	3.1	2.9
3	0.5	0.7	0.6	1.0	2.1	32	58	30	68	142	11	15	12	17	46	131	133	136	135	136	2.9	2.8	2.8	3.1	3.3
4	2.6	3.2	1.3	1.3	1.0	321	346	92	66	72	32	33	22	16	20	124	123	125	125	130	2.1	1.9	1.9	1.9	2.2
5	0.4	0.4	0.6	0.6	0.5	15	21	14	12	20	5	12	15	19	19	130	132	132	136	135	2.2	2.0	2.1	2.3	2.3
6	0.3	0.3	0.6	0.2	0.6	24	24	52	34	82	5	6	14	16	20	121	120	118	118	118	3.1	3.1	3.3	3.1	3.4
7	0.5	0.4	0.5	0.9	—	31	16	22	58	—	8	9	12	20	—	120	116	129	130	—	2.9	3.0	3.1	3.1	—
8	0.5	1.7	2.6	3.6	4.2	25	114	120	208	352	7	31	30	52	86	139	146	146	150	156	3.0	3.3	3.5	3.3	3.1
Mean	0.7	1.0	0.9	1.2	1.5	61	81	47	62	121	11	18	19	24	40	126	128	130	132	134	2.8	2.8	2.9	3.0	3.0
% of Control		143	129	171	214		133	77	102	198		164	173	218	364		102	103	105	106		100	104	107	107
P Value			.10	.50	.30	.20		.20	.50	.50	.40		.05	.10	.10	.05		.30	.05	.05	.10		.50	.30	.20	.05
Group B											*20 mg./kg. Rolicton given intravenously*														
1	0.5	1.3	0.9	2.2	2.5	29	139	74	188	186	16	26	20	29	37	130	133	132	138	137	3.3	2.7	3.0	3.0	3.4
2	3.2	6.5	3.6	3.5	3.9	348	514	252	236	324	42	49	34	34	40	127	120	140	142	140	2.1	2.0	2.2	2.4	2.4
3	0.7	2.2	4.6	2.2	3.0	60	173	484	294	432	8	21	57	43	63	137	141	141	141	140	2.8	3.5	3.2	3.1	3.2
4	1.1	1.4	1.2	2.4	3.7	47	49	44	162	320	14	14	18	47	71	138	143	137	145	144	2.8	2.4	2.6	3.1	3.5
5	0.5	0.6	0.7	1.0	1.2	24	12	6	54	124	15	19	18	26	32	132	133	137	142	140	3.1	2.3	2.2	3.0	3.0
6	1.5	4.7	6.1	6.0	3.3	248	342	398	386	212	42	49	39	37	29	131	136	142	145	149	2.3	2.1	2.6	1.9	2.7
7	2.1	2.6	4.0	4.8	3.5	196	158	238	228	145	25	26	27	27	23	126	132	134	134	136	2.0	1.8	1.7	2.0	2.2
8	1.0	1.5	2.8	3.4	3.8	124	210	314	392	436	21	24	48	54	68	131	133	133	137	135	2.6	2.5	2.6	2.7	2.6
Mean	1.3	2.6	3.0	3.2	3.1	135	200	226	243	272	23	29	33	37	45	132	134	137	141	140	2.6	2.4	2.5	2.7	2.9
% of Control		200	231	246	238		148	167	180	201		126	143	161	196		102	104	107	106		92	96	104	112
P Value			.05	.05	.01	.001		.05	.20	.05	.01		.01	.20	.10	.10		.30	.01	.001	.01		.50	.50	.50	.05

Key to abbreviations: See Table 2.

observation following drug administration.

Group III. In the animals receiving aminometradine (Mictine), there was a slight but temporary reduction in mean blood pressure at the 5 mg./kg. dose but no effect at the 20 mg./kg. dose. Glomerular filtration rate was not altered significantly (Table 5). As in the animals in Group II, initially there was a slight increase in renal blood flow (after 20 mg./kg.) followed by a reduction after 2 hours, probably a result of the prolonged anesthesia. There was a significant increase in water excretion ($P<0.01$) at both dosages within one hour after drug administration but the effect on sodium excretion appeared to be delayed somewhat (Table 6). The increase in water excretion reached a maximum of more than 300% of the control values 2 hours after drug administration employing the dose of 20 mg./kg. (Table 6*B*).

After administration of 30 mg. of aminometradine (Mictine) intravenously to 8 unhydrated dogs (Table 4) there was a significant increase in sodium and water excretion ($P<0.05$) which occurred within 30 minutes after drug administration and reached a maximum within 2 hours. The difference in the diuretic and natriuretic effects of the drug on the unhydrated dogs and the dogs on which simultaneous renal function studies were done is probably due to the differences in the state of hydration and urine output that existed prior to drug administration.

RESULTS OF CLINICAL BIOASSAY. The results of a previous study (Ford *et al.*[1]) revealed that aminophylline administered orally produced an increase in sodium excretion of only 41 mEq. per 24 hours at a dose of 1200 mg. and this was accompanied by gastrointestinal toxicity in over 25% of the patients. Thus, this drug would have a calculated potency estimation of less than 0.5 (when Mercuhydrin = 1).

The electrolyte excretion pattern (Fig. 1) following administration of 500 mg. of aminophylline given intravenously emphasizes the acute, short-lived action of this compound. The maximum effect of aminophylline was reached in the first 2-hour period after drug administration. Sodium and chloride excretion showed roughly equivalent increases of about nine times control level. There was a slight, but not significant increase in potassium and bicarbonate excretion. Ammonia and phosphate were slightly suppressed, but quickly returned to control level. In the second 2-hour period after the administration of aminophylline, sodium and chloride had decreased to about three times the control value. In the third 2-hour period, or 6 hours after drug administration, sodium and chloride were near control level where they remained for the subsequent three 6-hour periods.

Following the administration of aminoisometradine (Rolicton), 800 mg. as a single oral dose, there was an increase in sodium excretion of only 12 mEq./24 hours. Sixteen hundred milligrams produced an increase of 62 mEq. sodium per 24-hour period. The electrolyte excretion pattern following oral administration of 800 mg. of aminoisometradine (Rolicton) showed an equivalent increase in sodium and chloride excretion, with maximum natriuresis and chloruresis 6 to 12 hours after drug administration. Potassium excretion did not change significantly. There was a moderate increase in bicarbonate excretion. Ammonia and phosphate excretions were slightly depressed but quickly returned towards control values (Fig. 2).

An analysis of variance was performed on the data concerning aminoisometradine comparing it with meral-

TABLE 4.—DIURETIC RESPONSE TO AMINOMETRADINE & AMINOISOMETRADINE IN UNHYDRATED DOGS

		Urine Volume ml./min.							*Urine Sodium μEq./min.*							*Urine Potassium μEq./min.*						
Dog. No.	*Wt. kg.*	*C*	D_1	D_2	D_3	D_4	D_5	D_6	*C*	D_1	D_2	D_3	D_4	D_5	D_6	*C*	D_1	D_2	D_3	D_4	D_5	D_6
									Given 30 mg./kg. aminometradine (Mictine) intravenously													
1	12.0	0.2	0.2	0.3	2.7	2.0	0.6	0.3	20	27	40	377	323	133	83	2	6	20	46	46	19	15
2	9.0	0.2	0.2	0.2	0.3	0.4	0.5	—	19	40	27	40	53	67	—	10	15	18	23	24	26	—
3	12.0	0.3	0.4	0.6	0.9	1.3	1.7	1.4	50	67	100	150	217	227	227	5	7	7	10	17	23	25
4	13.0	0.6	0.8	0.7	0.6	0.6	0.4	0.3	50	113	113	107	110	93	87	16	20	11	10	5	4	5
5	9.0	0.2	0.5	0.4	0.4	0.5	0.4	0.4	17	27	20	17	20	17	20	6	8	8	6	7	8	9
6	13.0	0.1	0.3	0.3	0.3	0.5	0.4	0.3	35	50	63	60	87	87	77	13	14	21	16	19	18	9
7	11.0	0.1	0.2	0.2	0.3	0.3	0.2	0.2	13	17	20	40	60	57	47	12	16	23	29	31	21	18
8	9.0	0.1	0.2	0.2	0.2	0.2	0.2	0.1	25	40	33	20	33	33	27	5	11	9	7	9	5	3
Mean	11.0	0.2	0.4	0.4	0.7	0.7	0.6	0.4	29	48	52	101	113	89	81	9	12	15	18	20	16	12
% of Control			200	200	350	350	300	200		166	179	348	390	307	279		133	167	200	222	178	133
P Value* <			.05	.05	.20	.10	.20	.30		.05	.05	.10	.10	.05	.10		.001	.05	.20	.10	.05	.50
									Given 30 mg./kg. aminoisometradine (Rolicton) intravenously													
9	12.0	0.1	0.1	0.2	0.2	0.2	0.2	0.3	5	13	7	10	13	27	40	3	7	8	7	14	16	20
10	15.0	0.1	0.2	0.2	0.3	0.4	0.5	0.4	9	23	27	50	97	110	103	4	7	7	17	36	46	40
11	12.0	0.1	0.2	0.2	0.3	0.2	0.2	0.3	17	20	13	30	27	27	40	3	2	4	8	6	4	9
12	11.0	0.1	0.1	0.2	0.2	0.3	0.3	0.3	5	10	10	10	20	40	80	5	7	7	7	16	22	23
13	14.0	0.1	0.1	0.1	0.1	0.2	0.3	0.3	7	7	7	7	20	33	30	6	16	13	11	30	30	29
14	11.0	0.2	0.4	0.5	0.4	0.5	0.5	0.5	35	67	100	80	87	80	60	7	11	16	18	18	25	29
15	11.0	0.2	0.4	0.4	0.4	0.4	0.5	0.4	35	47	60	67	60	67	67	16	16	15	15	14	17	15
16	18.0	0.1	0.1	0.1	0.1	0.2	0.2	0.2	10	13	7	13	13	10	10	6	1	2	2	5	6	8
Mean	13.0	0.1	0.2	0.2	0.3	0.3	0.3	0.3	15	25	29	33	42	49	54	6	8	9	11	17	21	22
% of Control			200	200	300	300	300	300		167	193	220	280	327	360		133	150	188	283	350	367
P Value* <			.001	.001	.001	.001	.01	.001		.05	.20	.05	.05	.05	.01		.30	.10	.10	.05	.05	.05

C = Control observations: average of 2 thirty-minute urine collection periods.
D_1 to D_6 = Successive 30-minute urine collection periods after drug administration.

$$^{*}t = \bar{x}\sqrt{\frac{n(n-1)}{S_x 2}}$$

TABLE 3.—RENAL HEMODYNAMIC RESPONSE TO AMINOMETRADINE (MICTINE)

		Mean Blood Pressure mm.Hg					*Glomerular Filtration Rate ml./min.*					*Hematocrit*					*Renal Blood Flow ml./min.*				
Dog No.	*Wt. Kg.*	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4
Group A									*5 mg./kg. Mictine given intravenously*												
1	10.0	159	154	147	166	178	54	56	46	43	52	37	36	35	34	33	292	297	226	147	166
2	8.0	102	104	95	104	111	38	32	33	33	36	40	37	34	32	35	232	230	117	149	157
3	11.0	141	124	130	137	147	44	36	42	40	40	31	35	33	30	30	174	208	251	144	147
4	9.0	120	108	105	96	127	21	25	26	25	24	39	42	37	34	32	139	147	117	103	101
5	7.0	127	114	131	141	131	28	29	24	20	20	24	22	22	25	29	175	232	122	75	80
6	11.0	90	64	65	91	95	30	22	24	24	26	43	38	32	37	35	209	148	125	103	86
7	10.0	165	163	167	179	169	38	32	39	34	36	47	57	53	49	52	243	267	230	143	185
8	9.0	117	118	117	114	104	42	44	31	31	—	28	29	28	26	26	193	290	275	195	—
Mean	9.4	128	119	120	129	133	37	35	33	31	33	36	37	34	33	34	207	227	183	132	132
% of Control			93	94	101	104		95	89	84	89		103	94	92	94		110	88	64	64
P Value <			.05	.10	.50	.20		.30	.10	.01	.10		.50	.30	.05	.30		.30	.40	.01	.01
Group B									*20 mg./kg. Mictine given intravenously*												
1	9.0	169	172	169	164	170	30	34	35	36	40	41	45	40	35	36	185	227	165	128	138
2	9.0	126	120	111	111	125	30	34	27	24	33	20	23	20	20	18	148	168	144	90	106
3	12.0	111	103	102	117	128	18	15	17	18	14	37	36	37	37	43	116	130	119	116	91
4	10.0	108	95	98	107	111	38	38	34	31	32	40	44	42	33	42	205	205	195	113	136
5	13.0	115	110	118	83	133	48	49	52	51	49	43	39	38	44	40	249	215	202	227	170
6	15.0	91	91	106	103	107	52	56	45	69	53	44	48	46	40	43	279	300	243	272	196
7	13.0	118	109	124	127	148	34	27	35	40	37	45	46	43	41	42	189	241	168	169	159
8	14.0	114	118	119	125	137	51	56	58	48	60	47	45	44	43	41	349	371	321	263	249
Mean	11.9	119	115	118	117	132	38	39	38	40	40	40	41	39	37	38	215	232	195	172	156
% of Control			97	99	98	111		103	100	105	105		103	98	93	95		108	91	80	73
P Value <			.20	.50	.50	.05		.50	.50	.50	.40		.40	.30	.05	.20		.30	.05	.05	.001

Key: C to D_4: See Table 2.

TABLE 6.—THE EFFECT OF AMINOMETRADINE (MICTINE) ON WATER AND ELECTROLYTE EXCRETION

	Urine Volume ml./min.					*Urine Sodium μEq./min.*					*Urine Potassium μEq./min.*					*Plasma Sodium mEq./L.*					*Plasma Potassium mEq./L.*				
Dog No.	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4	*C*	D_1	D_2	D_3	D_4
Group A											*5 mg./kg. Mictine given intravenously*														
1	0.5	0.9	0.5	0.7	0.9	4	5	2	3	6	11	30	16	22	31	130	130	134	134	137	—	—	—	—	—
2	0.5	0.6	0.8	1.4	1.6	8	6	11	13	18	14	19	21	22	31	129	132	133	133	—	2.4	2.6	2.1	—	—
3	0.5	0.6	2.3	1.2	2.4	6	5	20	7	13	11	19	29	19	26	130	132	138	140	144	2.8	2.6	2.0	2.0	2.5
4	0.4	0.7	1.5	1.3	1.4	5	7	15	12	12	7	19	22	21	21	125	126	131	130	130	2.2	2.4	2.7	2.4	2.3
5	0.5	0.7	0.8	0.6	0.4	39	42	42	40	44	18	18	16	10	16	130	138	138	138	140	2.5	3.3	3.9	4.7	3.5
6	0.8	1.2	1.1	1.7	2.3	88	94	70	88	100	15	14	10	6	12	142	134	132	136	141	2.9	2.5	2.3	2.5	2.5
7	0.5	0.5	0.6	0.4	1.5	85	64	42	42	100	6	9	12	12	22	126	134	140	144	142	3.0	2.9	2.9	3.7	3.1
8	0.5	0.3	0.6	0.4	—	26	22	24	22	20	3	7	4	11	4	136	138	138	134	126	2.4	2.7	2.5	2.9	3.0
Mean	0.5	0.7	1.0	1.0	1.5	33	31	28	28	39	11	17	16	15	20	131	133	136	136	137	2.6	2.7	2.6	3.0	2.8
% of Control	140	200	200	300			94	85	85	118		155	145	136	182		102	104	104	105		104	100	115	108
P Value <		.05	.01	.05	.01		.50	.50	.50	.01		.05	.10	.30	.05		.30	.20	.10	.10		.50	.50	.40	.40
Group B											*20 mg./kg. Mictine given intravenously*														
1	0.4	0.5	0.6	0.8	1.7	44	50	36	72	198	11	21	12	23	44	135	137	135	136	141	2.6	2.4	2.5	3.2	2.3
2	0.4	0.4	0.5	0.6	1.1	43	30	24	22	60	7	11	13	12	30	129	134	137	142	146	2.3	2.2	2.9	3.1	3.3
3	0.6	0.5	0.8	1.2	1.3	31	16	22	48	62	15	12	16	18	23	134	131	133	140	137	2.7	2.4	2.4	2.9	2.8
4	1.1	1.5	1.7	1.4	1.2	159	98	38	26	24	19	21	20	19	22	133	139	134	138	141	2.7	2.2	2.4	2.5	2.4
5	0.4	0.4	0.7	3.1	2.3	47	44	54	146	116	18	23	25	39	40	128	134	132	130	131	3.6	3.3	3.7	3.5	3.1
6	0.4	0.9	1.9	3.5	3.1	32	34	24	128	168	24	27	17	32	44	127	126	132	138	138	—	—	—	—	—
7	0.7	0.6	1.0	2.5	4.0	91	44	56	170	260	14	18	19	29	38	139	134	131	132	136	2.5	2.3	2.5	2.6	3.1
8	0.7	1.3	2.6	2.4	2.8	20	80	84	58	134	22	37	31	28	32	128	128	128	131	134	—	—	—	—	—
Mean	0.6	0.8	1.2	1.9	2.2	58	50	42	84	128	16	21	19	25	34	132	133	133	136	138	2.7	2.5	2.7	3.0	2.8
% of Control	133	200	317	367			86	72	145	221		131	119	156	213		101	101	103	105		93	100	111	104
P Value <		.10	.05	.05	.01		.40	.30	.50	.01		.05	.20	.01	.01		.50	.50	.10	.05		.01	.20	.50	.50

Key: C to D_4: See Table 2.

luride, a standard diuretic. The two dosage response curves were parallel, and the error in the experimental trials was not significant. A calculation of potency estimation based on sodium excretion was done. This showed aminoisometradine to be 0.5 times as potent following oral administration as an increase in sodium excretion of 38 mEq./24 hours and a dose of 1200 mg. produced an increase in sodium excretion of 89 mEq./24 hours (Table 7). Since aminoisometradine (Rolicton) is an isomer of aminometradine (Mictine) the electrolyte excretion pattern of aminometradine (Mictine) was presumed to be like that just described for aminoisometradine (Rolicton) and was not observed.

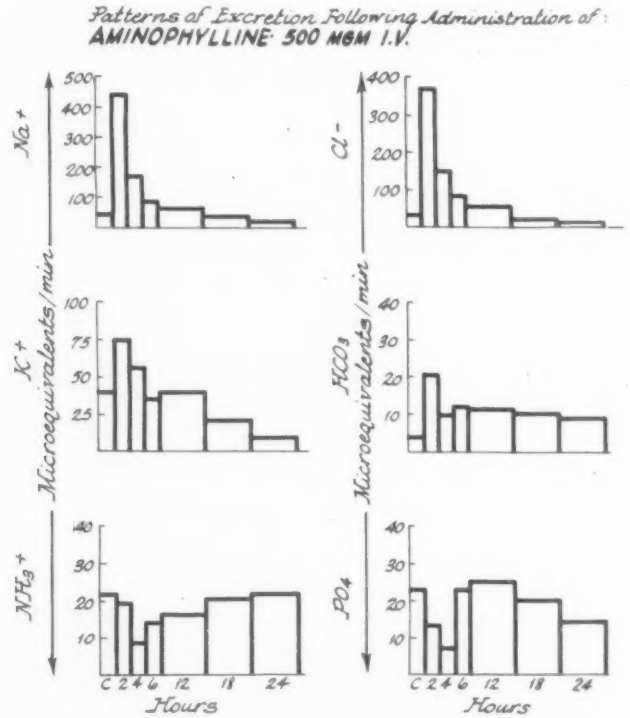

Fig. 1.—The electrolyte excretion pattern following the administration of 500 mg. of aminophylline intravenously. There is a significant increase in sodium and chloride excretion which, however, is only of short duration.

meralluride following parenteral administration. Or, 1598 mg. of aminoisometradine (Rolicton) orally was equivalent in ability to increase sodium excretion to 40 mg. Hg (1 ml.) meralluride (Mercuhydrin) intramuscularly (Fig. 3).

A single oral dose of 600 mg. of aminometradine (Mictine) produced

An analysis of variance comparing aminometradine with the standard meralluride, showed that the dosage curves for sodium were parallel, and the error nonsignificant. With meralluride as one, aminometradine (Mic-

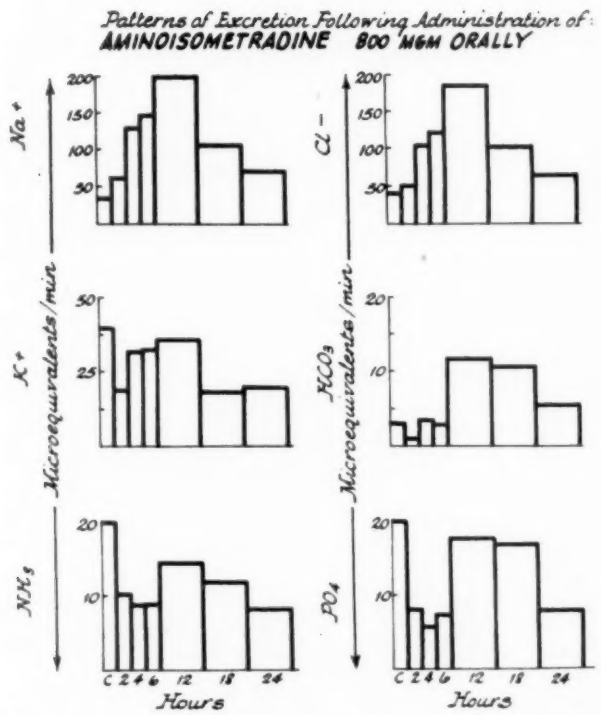

Fig. 2.—The electrolyte excretion patterns following the administration of 800 mg. of aminoisometradine as a single oral dose. This response is similar to that of aminophylline given intravenously but it is less rapid in onset and of greater duration.

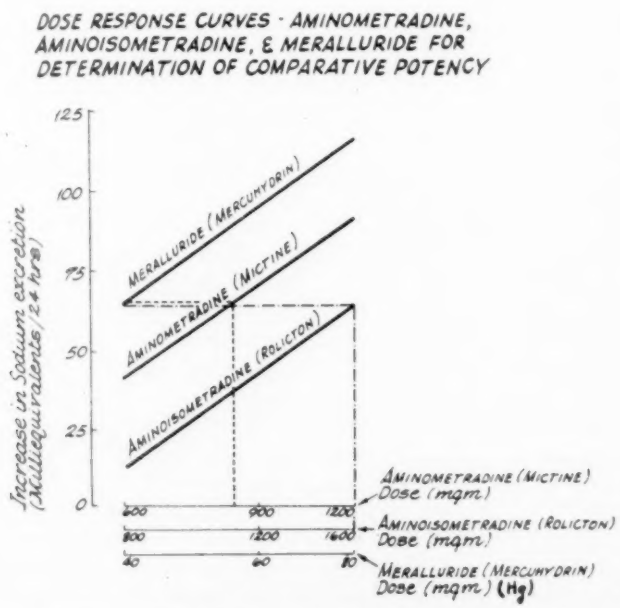

Fig. 3.—Dosage response curves for aminometradine, aminoisometradine, and meralluride showing their comparative potency.

tine) was 0.7 times as potent as meralluride, or 856 mg. of orally administered aminometradine (Mictine) produces an increase in sodium excretion equivalent to 40 mg. Hg (1 ml.) meralluride (Mercuhydrin) (Fig. 3).

TABLE 7.—EFFECTS OF AMINOISOMETRADINE AND AMINOMETRADINE ON SODIUM EXCRETION

Aminoisometradine

800 mg.				*1600 mg.*		
C	*D*	*I*		*C*	*D*	*I*
50	55	5		45	103	58
45	53	8		50	120	70
50	70	20		45	105	60
48	64	16		50	98	48
43	50	7		45	104	59
50	64	14		49	108	59
44	57	13		50	115	65
50	56	6		45	106	61
50	58	8		42	112	70
45	68	23		48	115	67
—	—	—		—	—	—
48	60	12	Average	47	109	62

Aminometradine

600 mg.				*1200 mg.*		
C	*D*	*I*		*C*	*D*	*I*
44	90	46		46	161	115
48	102	54		49	181	132
44	90	46		43	146	103
46	66	20		45	120	75
43	69	26		47	130	83
47	86	39		46	166	120
45	83	38		42	86	44
42	67	25		44	110	66
49	105	56		46	160	114
40	66	26		40	77	37
—	—	—		—	—	—
45	83	38	Average	45	134	89

C = Control, mEq. sodium excretion/24 hrs.
D = Drug, mEq. sodium excretion/24 hrs.
I = Increase, mEq. sodium excretion/24 hrs.

Discussion. The aminouracil diuretics are derivatives of the pyrimadine uracil which is found in nucleic acid. In aminometradine (Mictine), a methallyl group has been substituted at the one position of uracil and a methyl group at the 3 position. Rolicton is the isomer of Mictine. Aminophylline, the most important of the xanthine diuretics, is a purine derivative and is also found in nucleic acid. Aminophylline is the 1,3-methyl derivative of the purine base, xanthine (Fig. 4).

Aminophylline has long been known to exert effects of broncho-dilatation, coronary dilatation, increased renal blood flow and cardiac output. However, our previous experience with this drug in dogs showed no alteration of glomerular filtration rate. Likewise, neither aminoisometradine (Rolicton) nor aminometradine (Mictine) showed any alteration in glomerular filtration rate in dogs.

Aminophylline, aminoisometradine (Rolicton) and aminometradine (Mictine) have similar effects on renal transport mechanisms for various electrolytes. All three caused increased excretion of sodium and chloride, slight inhibition of ammonia and phosphate excretion without consistent changes in potassium and bicarbonate excretion. The maximum effects of intravenously administered aminophylline occurred acutely within 2 hours. Aminoisometradine (Rolicton) and aminometradine (Mictine), after oral administration, exhibited their maximum effects within 6 to 12 hours.

Aminophylline, due to its acute, short-lived action, following intravenous administration finds its greatest use in acute states of cardiac decompensation. It may be useful for enhancing the action of mercurial diuretics. Aminoisometradine (Rolicton) and aminometradine (Mictine) are both useful as oral diuretics but are only moderately potent and are indicated in the treatment of patients with mild heart failure or for prolonging the period between injections of the organomercurials in patients with moderately severe to severe heart failure.

Aminophylline is less than 0.5 times as potent as meralluride (Mercuhy-

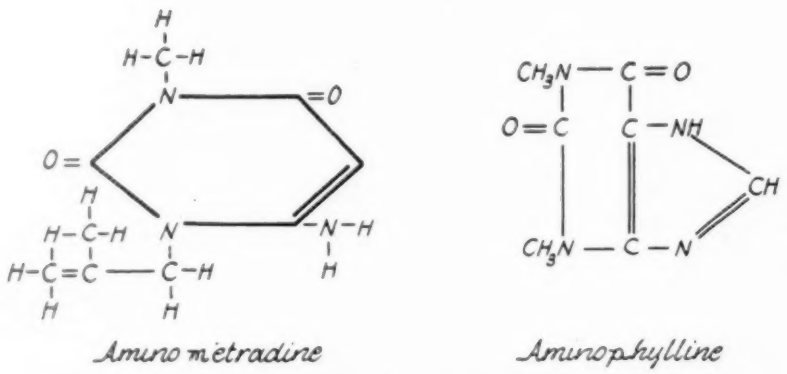

Fig. 4.—The structural formulae of aminometradine and aminophylline.

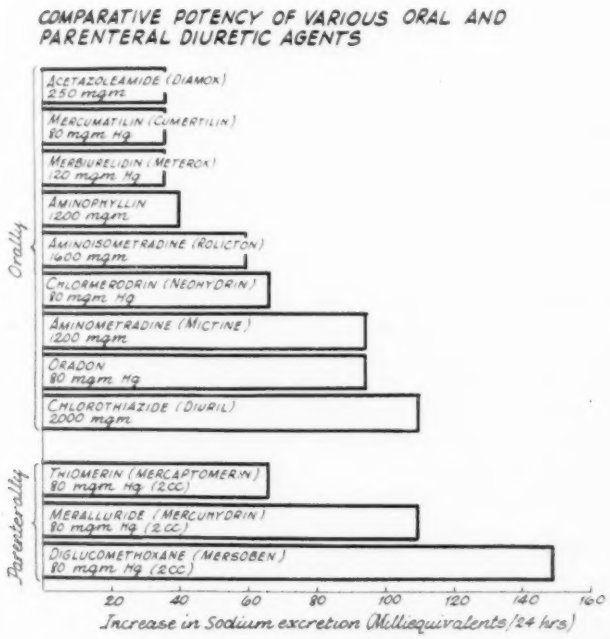

Fig. 5.—The comparative potency of various oral and parenteral diuretic agents is graphically demonstrated in terms of the ability to augment sodium excretion at maximum tolerated doses.

drin). Aminometradine (Mictine) is 0.7 times, and aminoisometradine (Rolicton) is 0.5 times as potent following oral administration as parenteral meralluride. A table of comparative potency estimations illustrates the appropriate rank of these compounds (Table 8). This is graphically demonstrated in terms of the ability to augment sodium excretion at the dogs, aminophylline, aminoisometradine (Rolicton) and aminometradine (Mictine) all produced a significant increase in sodium and water excretion.

In humans, parenteral aminophylline and orally administered aminoisometradine (Rolicton) and aminometradine (Mictine) all produced moderately increased sodium excretion. Orally administered aminophylline is less than

TABLE 8.—COMPARATIVE POTENCY ESTIMATIONS

Type of Diuretic	*Specific Drug*	*Method of Administration*	*Potency Estimation*
Organomercurial	chlormerodrin (Neohydrin)	P	3.3
	chlormerodrin (Neohydrin)	O	0.5
	meralluride (Mercuhydrin)	P	1.0
	thiomerin (Mercaptomerin)	P	0.6
	mercumatilin (Cumertilin)	O	<0.5
	merbiurelidin (Meterox)	O	<0.5
	diglucomethoxane (Mersoben)	P	1.3
	WY 1204 (Oradon)	O	0.7
Carbonic anhydrase inhibitor	acetazolcamide (Diamox)	O	<0.5
	ethoxzolamide (Cardrase)	O	<0.5
	SKF 4965 (Butamide)	O	<0.5
	Dirnate	O	<0.5
Xanthine	aminoisometradine (Rolicton)	O	0.5
	aminometradine (Mictine)	O	0.7
	Aminophylline	O	<0.5
	Aminophylline	P	<0.5
Acidifying salts	Ammonium chloride (alone)	O	<0.5
"Chlorothiazide"	chlorothiazide (Diuril)	O	0.8
	chlorothiazide (Diuril)	P	<0.5

Method of Administration: P = parenterally (i.v. or i.m.) O = orally
Potency Estimation: Comparative response in sodium excretion using meralluride (Mercuhydrin) as "1."

highest clinically acceptable dosages in Fig. 5. This also includes similar results for other classes of diuretic agents.

Conclusions. In hydrated dogs, aminophylline and aminoisometradine (Rolicton) given intravenously produces a significant increase in sodium and water excretion. Intravenous aminometradine (Mictine) caused a moderate diuresis in hydrated dogs but less natriuresis than in the case of aminoisometradine. In unhydrated 0.5 times as potent as meralluride (Mercuhydrin) intramuscularly administered. Orally administered aminoisometradine (Rolicton) is 0.5 times and aminometradine (Mictine) is 0.7 times as potent as meralluride (parenteral).

Thus, aminometradine (Mictine) and aminoisometradine (Rolicton), in that order of potency, add two more orally effective agents for use in the treatment of chronic states of edema.

REFERENCES

1. **Ford, R. V., Handley, C. A., Moyer, J. H.,** and **Spurr, C. L.:** J. Antibiotic Med. and Clin Ther., in press.

2. **Ford, R. V., Moyer, J. H.,** and **Spurr, C. L.:** A.M.A. Arch. Int. Med., in press.
3. **Ford, R. V., Spurr, C. L.,** and **Moyer, J. H.:** J. Antibiotic Med. and Clin. Ther., in press.
4. Iidem: Circulation, in press.
5. **Handley, C. A., Chapman, D.,** and **Moyer, J. H.:** Proc. Soc. Exper. Biol. & Med., **78,** 433, 1951.
6. **Handley, C. A., Sigafoos, R. B.,** and **LaForge, M.:** Am. J. Physiol., **159,** 175, 1949.
7. **Moyer, J. H., Ford, R. V., Handley, C. A.,** and **Spurr, C. L.:** To be published.
8. **Moyer, J. H.,** and **Handley, C. A.:** J. Pharmacol. & Exper. Therap., **113,** 383, 1955.

SUMMARIO IN INTERLINGUA

Observationes Laboratorial e Clinic Relative a Tres Simile Agentes Diuretic: Aminophyllina, Aminometradina (Mictina), e Aminoisometradina (Rolicton)

In canes hydratate, le administration intravenose de aminophyllina e aminoisometradine (Rolicton) produce significative augmentos del excretion de natrium e aqua. Le administration intravenose de aminometradina (Mictina) causa in tal canes moderate grados de diurese, sed le natriurese assi effectuate es minus que in le caso de aminoisometradina. In canes non subjicite a hydratation, aminophyllina, aminoisometradina (Rolicton), e aminometradina (Mictina) produceva omnes augmentos significative del excretion de natrium e de aqua.

In humanos, aminophyllina parenteral e administrationes oral de aminoisometradina (Rolicton) e de aminometradina (Mictina) produceva omnes augmentos moderate del excretion de natrium. Le potentia de aminophyllina in administrationes oral es minus que 0,5 vices le potentia de merallurido (Mercuhydrina) in administrationes intramuscular. Le potentia de aminoisometradina (Rolicton) in administrationes oral es 0,5 vices e illo de aminometradina (Mictina) es 0,7 vices le potentia de merallurido (Mercuhydrina) in administrationes parenteral.

Assi aminometradina (Mictina) e aminoisometradina (Rolicton), in iste ordine secundo lor potentia, representa duo agentes additional de efficacia post administrationes oral in le tractamento de statos chronic de edema.

PAPER ELECTROPHORETIC PARTITION OF SERUM PROTEIN AND C-REACTIVE PROTEIN IN PATIENTS WITH BRONCHIECTASIS WITH A PRELIMINARY REPORT ON THE EFFECT OF PROLONGED ANTIBIOTIC TREATMENT*

By Kenneth L. Vosti, M.D.
RESEARCH ASSISTANT

Joyce Z. Pearson, M.D.
RESEARCH ASSISTANT

Mark H. Lepper, M.D.
PROFESSOR OF PREVENTIVE MEDICINE

Harry F. Dowling, M.D.
PROFESSOR OF MEDICINE

AND

George G. Jackson, M.D.
ASSOCIATE PROFESSOR OF MEDICINE

(From the Departments of Preventive Medicine and Medicine, University of Illinois, Chicago, Illinois)

As part of a study in which an evaluation of the prolonged use of antibiotics in the treatment of bronchiectasis is being made, different moities have been chosen in an attempt to measure the response to therapy. The observations of others (Aaronson *et al.*[1], Baldwin and Hand[2], Berlin, Wallace and Meyer[3], Bing[4], Elster, Braundwald and Wood[7], Gilliland *et al.*[8], Hedlund[11], Kagan[14], Kaplan *et al.*[15], Malmros and Blix[16], Roantree and Rantz[19], Selman and Halperin[20], and Yocum and Doerner[22]) that the distribution of serum proteins is abnormal and that C-reactive protein circulates in many patients with acute and chronic inflammatory pulmonary disease suggested that these might serve as useful indices of some of the systemic manifestations of the infection in the bronchi and lungs. Should return of abnormal values toward the normal range occur, it would indicate mitigation of the process.

It is the purpose of this paper to present the results of serial protein determinations, their distribution upon paper electrophoresis, and serologic C-reactive protein values on 29 patients with bronchographic evidence of bronchiectasis. Included in this group were 2 patients with hypogammaglobulinemia. For comparison, data on 2 other patients with hypogammaglobulinemia and recurrent pulmonary infections without bronchiectasis are reported.

Material and Methods. Only patients in whom bronchograms revealed bronchiectasis were accepted for this study. Most were not considered candidates for surgical resection because of the extent of the disease or the refusal to accept the procedure. Age, race,

*This study was generously supported by a grant from the Tuberculosis Institute of Chicago and Cook County and the Charles Pfizer Company, Brooklyn, New York.

and sex distribution are seen in Table 1. After an initial observation period, the patients were divided into 4 balanced groups and in a double blind manner were given either a placebo, 400,000 units of crystalline penicillin, 500 mg. of tetracycline, or 500 mg. of a chemical combination of penicillin and oleandomycin 4 times a day†. Observation periods, thus far, range from one to 8 months and are broken into pretreatment and post-treatment periods. The chemical determinations reported in this paper were performed several times on each patient before assignment to a treatment group and at monthly intervals after the initiation of therapy. Other studies which have been made in the pre- and post-treatment periods have included multiple pulmonary function tests and repeated sputum cultures on a variety of media. These results will be the subject of other reports.

TABLE 1.—AGE, SEX, RACE AND ASSOCIATED DISEASES IN 29 PATIENTS WITH BRONCHIECTASIS

Age: Mean 51.6 years, range 18 to 73 years
Sex: 20 males, 9 females
Race: 26 white, 3 Negro
Associated disease:

	No. Patients
Diabetes	2
Hepatosplenomegaly and eosinophilia, cause unknown	1
Pulmonary tuberculosis, inactive	1
Rheumatoid arthritis	1
Syringomyelia	1

Total proteins were determined by the Biuret method with normal range 6 to 8 gm. per 100 ml. of serum. Electrophoretic determinations were done by application of 0.01 ml. of serum to filter paper wicks that were run at 4 ma. for 20 hours on a Spinco Model R apparatus. The tests were performed in a constant temperature (72° F.) room with constant humidity of about 40%. A 0.075 ionic strength barbiturate solution buffered to pH 8.6 was used. The wicks were stained in a Bromphenol blue zinc sulfate dye for 6 hours; rinsed for 6 minutes twice in 5% acetic acid bath; bathed in a fixative consisting of 9 gm. sodium acetate (3 H_2O) made up to one liter with water for 6 minutes; and then dried at 120 to 130° C. for 15 minutes. Following exposure to ammonium hydroxide vapors for one minute, they were analyzed on a Spinco Analytrol Scanner and Integrater. Normal values were obtained initially from individual sera of 7 laboratory personnel ranging in age from 23 to 45.

C-reactive protein determinations were done by the rabbit antiserum precipitin technique. Commercial C-reactive protein rabbit antiserum and the patient's serum, 1.5 cm. of each, were drawn into a capillary tube (external diameter about 1 mm.) and incubated for 2 hours at 37° C. The degree of precipitation was read at approximately 24 hours following overnight refrigeration at 4° C.

Results. The total protein content of serum was measured in 29 patients. Sixty-eight per cent of the values ranged from 7 to 8 gm./100 ml. (Table 2). Only 2 sera were between 6 and 6.5 gm./100 ml. and both of these patients had hypogammaglobulinemia.

TABLE 2.—DISTRIBUTION OF VALUES FOR TOTAL PROTEIN CONTENT OF SERUM IN PATIENTS WITH BRONCHIECTASIS

Protein gm./100 ml.	*Patients*	
	Number	*%*
6.0 to 6.5	2*	8
6.6 to 7.0	3	12
7.1 to 8.0	17	68
8.1 to 8.4	3	12
Totals	25	100

* Both have hypogammaglobulinemia

The distribution of the protein fractions obtained after separation by paper electrophoresis are shown in Fig. 1. The mean normal values and 2 standard deviations above or below it

†Antibiotics were kindly supplied by Charles Pfizer and Company, Brooklyn, N.Y.

are represented by the shaded area. Each determination for the 27 patients with bronchiectasis was represented by a closed circle and the mean of these by the solid line. The concentrations measured on sera from the patients with bronchiectasis who also had hypogammaglobulinemia were indicated in the Figure but not included in the calculation of the mean for the bronchiectasis group. In Table 3 it may be seen that only the mean depression in albumin was significantly different from the normal value by the usually accepted standards. The mean percentages of α_2 and γ-globulin were somewhat elevated. However, as is seen in Fig. 1, 33% of the individual γ-globulin and 19% of the α_2 globulin determinations were higher than the upper limit of two standard deviations above the normal mean. When the ages of the patients were considered, the distribution of the protein fractions was the same among the patients over 50 as among those under 50 years of age. When the electrophoretic patterns on the 4 patients with hypogammaglobulinemia were evaluated separately and as a group, little difference was

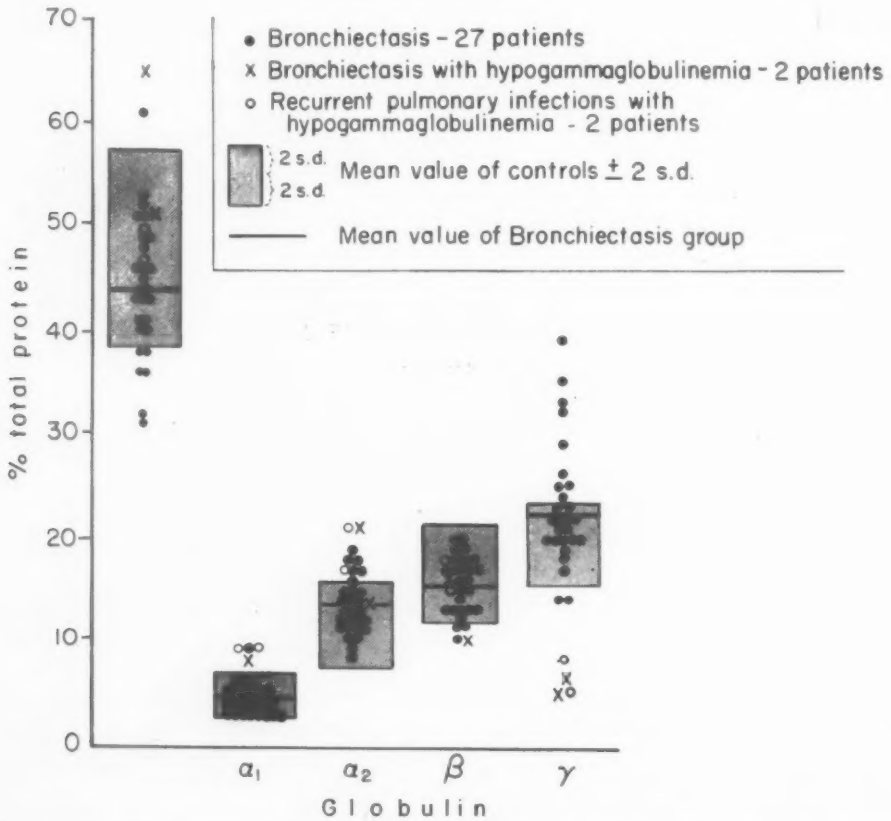

Fig. 1.—Results of serum protein fractionation by paper electrophoresis in 27 patients with bronchiectasis.

revealed between those from patients with or without bronchiectasis (Table 4). Most noticeable was the almost complete absence of γ-globulin. Other changes were elevations of the α_1 and α_2-globulin percentages in 3 of the 4 patients. In spite of the lower total protein concentrations among the patients, these changes represent absolute increases in the total amounts of these fractions.

made for the different components measured. The fractions remained equivalent in all groups except in the group treated with penicillin in which there was a significant decrease in the α_1-globulin proportion. No significant changes in the γ-globulin fractions were found.

Analysis of the pre- and post-treatment C-reactive protein data likewise failed to show a statistically significant

TABLE 3.—DISTRIBUTION OF ELECTROPHORETICALLY SEPARATED SERUM PROTEIN FRACTIONS FOR NORMAL AND BRONCHIECTATIC PATIENTS

	Normals			*Bronchiectasis*			
	Mean % of Total Protein	*S. D.*	*S. E.*	*Mean % of Total Protein*	*S. D.*	*S. E.*	*T. Values*
Albumin	48	±4.8	±1.10	44.1	±6.5	±1.20	2.28
α_1-globulin	4.7	1.1	.25	4.5	1.4	.27	.51
α_2-globulin	11.7	2.3	.53	13.3	2.8	.54	1.97
β-globulin	16.8	2.6	.59	15.5	2.8	.54	1.54
γ-globulin	19.6	1.9	.44	22.4	6.0	1.10	1.74

S. D. = Standard deviation
S. E. = Standard error

TABLE 4.—DISTRIBUTION OF ELECTROPHORETIC SERUM PROTEIN FRACTIONS (% TOTAL PROTEIN) IN PATIENTS WITH HYPOGAMMAGLOBULINEMIA

	*G.**	*Z.**	*P.†*	*W.†*
Albumin	65%	51%	47%	50%
α_1-globulin	6%	8%	9%	9%
α_2-globulin	14%	21%	21%	17%
β-globulin	10%	16%	18%	15%
γ-globulin	6%	5%	5%	8%

* Bronchiectasis
† Recurrent pulmonary infections with no evidence of bronchiectasis

C-reactive proteins which were measured serially on sera obtained from 26 patients are shown in Table 5. In only 2 patients were the reactions persistantly negative, while in 2 others the reactions were frequently negative. Also of interest was the fact that this protein was present in both patients who had bronchiectasis and hypogammaglobulinemia as well as in one of the 2 with recurrent pulmonary infections.

Comparison of mean values of the pre- and post-treatment periods were change toward lower degrees of reactivity. In the group treated with a combination of penicillin and oleandomycin there was a suggestive return toward normal, but when submitted to chi square analysis this difference was not significant.

Discussion. A depression in the serum albumin concentration and an elevation in the γ-globulin fractions have been noted in a variety of pulmonary diseases, particularly those of a chronic nature (Baldwin and Hand[2], Bing[4], Gilliland *et al.*[8], Kagan[14], and

Malmros and Blix[16]). Bronchiectasis is such a disease, but even though the changes in this study were consistent with these reports, they were small and generally of little consequence. though reciprocal depression in association with hyperglobulinemia (Bing[4]) and malnutrition (Kagan[14]) has been suggested as a causative factor. The elevation of γ-globulin is believed to

TABLE 5.—SERIAL SERUM C-REACTIVE PROTEIN DETERMINATIONS IN PATIENTS WITH BRONCHIECTASIS

Treatment groups	*Oct.*	*Nov.*	*Dec.*	*Jan.*	*Feb.*	*Mar.*	*Apr.*	*May*
Placebo (6 patients)								
1	0	0	0	0	0	2	0	0
2				2	2	3	4	4
3						>1 1	3	>1
4	2	1		1		1	1	1
5	1		2		2	1	2	>1
6			2			1	4	1
Tetracycline (6 patients)								
1	1	2		3 2	1	5	3	1
2			1,1	2	4	1	1	>1
3	>1	1 >1	>1	>1	>1		2	0
4	1	4	>1	1	0	1	1	0
5	0	0	>1	0	>1	0	>1	0
6	0	>1,2	1				1	0
Penicillin-Oleandomycin (6 patients)								
1	4	2		5	1			2
2		1			3	1	1	
3		5	1	0	0	0	>1	0
4	>1	1 4		>1		0		
5	1,1	1 2	1	1	1	1	>1	>1
6	0	0	0	0	0	0	0	0
Penicillin (8 patients)								
1	4		1	2	2	2	1	1
2					0	0	0	
3						1	1	1
4	>1	0,0	>1	4	>1	0	1	
5				>1	0	0	2	
6				2,2	4	1	2	
7			6	2	1	2	3	3,4
8	>1,>1	>1,>1	1		1	>1	>1	0

The values preceding the solid line are in the pre-treatment period, and those following the solid line are in the post-treatment period.

The α_2-globulin, which has also been reported as elevated in infection (Baldwin and Hand[2], Gilliland *et al.*[8], and Malmros and Blix[16]), tended to be elevated in our patients. Serum albumin changes have been unexplained, al- represent antibody production by the reticuloendothelial system in response to infection (Berlin, Wallace and Meyer[3] and Bing[4]). Several authors (Baldwin and Hand[2] and Gilliland *et al.*[8]) have reported success in using

the reversion of these changes toward normal as guides to response to therapy in pulmonary tuberculosis where albumin depression and α_2 and γ-globulin elevations have also been noted. We had hoped that similar serial analysis would allow an evaluation of the disease in patients with bronchiectasis who were placed on prolonged antibiotic therapy. The small mean changes, however, were not significant. Therefore, individual patients who had initial values well beyond the normal range were analyzed separately. Even when this was done there was no consistent evidence of improvement. It is believed that this may have been the result of the shortness of the observation period and the paucity of patients in each group.

In addition to the measurements made by electrophoresis the occurrence of an abnormal protein (C-reactive protein) in the presence of inflammation has been noted in a variety of conditions (Aaronson *et al.*[1], Elster, Braundwald and Wood[7], Hedlund[11], Kaplan *et al.*[15], Roantree and Rantz[19], Selman and Halperin[20], and Yocum and Doerner[22]). It, too, was investigated as of potential worth for serial evaluation. Its presence in almost all of our patients was interpreted as a reflection of the activity of the pulmonary disease. In only a few of the patients were there any other factors which could have contributed. The degree of positivity was uncorrelated with any clinical observations such as sputum volume, or character, or bacteria isolated from the sputum. When acute respiratory disease supervened, the highest values were obtained. When the pretreatment were compared with the post-treatment values, no significant changes were apparent. Again the limiting factors may be the short post-treatment period and the small groups.

The occurrence of recurrent pulmonary disease and bronchiectasis with agammaglobulinemia has been previously noted. Good and Mazzitello[10] reviewed the literature and found that among 24 patients with congenital and 19 with acquired agammaglobulinemia, all but 6 had some type of pulmonary disease. Four of the congenital group and 4 of the acquired group had bronchiectasis. In this study 4 additional patients were observed whom we considered to have the acquired form. Two of these had extensive, bilateral bronchiectasis in addition to recurrent pulmonary infections. The unusual susceptibility of these patients to infection is believed to be associated with inability of the reticuloendothelial system to form antibodies. In addition to the low or absent γ-globulin Gitilin, Hitzig and Janeway[9] have recently been able to demonstrate multiple serum protein deficiencies in the form of 2 plasma proteins that migrate with the β-globulins in agar. Our patients all had low γ-globulin levels and in addition 3 of 4 had elevated α_1 and α_2-globulin fractions. The literature contains little information about these fractions other than occasional comment that all fractions other than γ-globulin were normal. Whether these changes are relative to the absence of γ-globulin or are a secondary response to infection cannot be definitely stated.

The presence of C-reactive protein has been noted previously in several patients with agammaglobulinemia. This observation recently caused Roantree, Pezold and Rantz[18] to re-evaluate its migrating position by continuous flow electrophoresis. Their observations that it occurred in the γ-globulin fraction confirmed those of Hedlund[12] but were in contrast to those of Perlman, Bullowa and Goodkind[17] who felt that it migrated with the α_1-globulin. It was interesting that 3 of our 4 patients with hypogammaglobu-

linemia had C-reactive protein in their sera, one in quite high values. This can be interpreted in 2 ways. C-reactive protein is not a gamma globulin or that even though it migrates with the gamma fraction it is still present. In the latter case it may be produced by different cells than the other gamma globulins or its presence indicates that, as is usually true, gamma globulin is not entirely absent.

Summary. 1. Various serum protein determinations were done in 29 patients with bronchiectasis.

2. Patients with bronchiectasis showed significant mean depression in albumin and elevations of the mean values for α_2 and γ-globulin fractions of borderline significance.

3. C-reactive protein was present in the sera at some time in almost all of the patients.

4. Pre- and post-treatment comparison of the electrophoretic patterns and C-reactive protein failed to reveal a significant change.

5. Four additional patients with hypogammaglobulinemia and recurrent pulmonary disease including 2 with bronchiectasis were presented. In addition to the almost complete absence of γ-globulin, 3 patients had elevations in α_1 and α_2-globulins and C-reactive protein.

REFERENCES

1. **Aaronson, A., Kaplan, M., Goldin, M., Libretti, A.,** and **Goldman, B.:** Ann. Allergy, **13,** 586, 1955.
2. **Baldwin, R.,** and **Hand, C.:** Am. Rev. Tuberc., **68,** 372, 1953.
3. **Berlin, I., Wallace, S.,** and **Meyer, L.:** A.M.A. Arch. Int. Med., **85,** 144, 1950.
4. **Bing, J.:** Acta med. Scandinav., **103,** 547, 1940.
5. **Collins, H.,** and **Dudley, H.:** New England J. Med., **252,** 255, 1955.
6. **Davenport, C.,** and **Ekas, M.:** Statistical Methods in Biology, Medicine and Psychology. New York: John Wiley and Sons, Inc., 1936.
7. **Elster, S., Braundwald, E.,** and **Wood, H.:** Am. Heart J., **51,** 533, 1955.
8. **Gilliland, I., Johnston, R., Stradling, P.,** and **Abdel-Wahab, E.:** Brit. Med. J., **1,** 1460, 1956.
9. **Gitilin, D., Hitzig, W.,** and **Janeway, C.:** J. Clin. Invest., **35,** 11, 1956.
10. **Good, R.,** and **Mazzitello, W.:** Dis. Chest, **29,** 9, 1956.
11. **Hedlund, P.:** Acta med. Scandinav., Suppl., **196,** 579, 1947.
12. Idem: Scandinav. J. Clin. & Lab. Invest., **7,** 99, 1955.
13. **Hiller, A.:** Practical Clinical Chemistry. Springfield, Ill.: Charles C Thomas, 1953.
14. **Kagan, B.:** Am. J. Med. Sci., **206,** 309, 1943.
15. **Kaplan, M., Aaronson, A., Henderson, J.,** and **Goldin, M.:** Ann. Allergy, **13,** 29, 1955.
16. **Malmros, H.,** and **Blix, G.:** Acta med. Scandinav., Suppl., **170,** 280, 1946.
17. **Perlman, E., Bullowa, J.,** and **Goodkind, R.:** J. Exper. Med., **77,** 97, 1943.
18. **Roantree, R., Pezold, F.,** and **Rantz, L.:** Clin. Res. Proc., **5,** 159, 1957.
19. **Roantree, R.,** and **Rantz, L.:** A.M.A. Arch. Int. Med., **96,** 674, 1955.
20. **Selman, D.,** and **Halperin, A.:** Angiology, **7,** 292, 1956.
21. **Seltzer, G., Baron, S.,** and **Toparek, M.:** New England J. Med., **252,** 252, 1955.
22. **Yocum, R.,** and **Doerner, A.:** A.M.A. Arch. Int. Med., **99,** 74, 1957.
23. **Zinnernan, H., Hall, W.,** and **Heller, B.:** J. Am. Med. Assn., **156,** 1390, 1954.

(Interlingua on page 677)

EXPERIMENTAL CEREBRAL INFARCTION: THE EFFECT OF DICUMAROL*

By William A. Sibley, M.D.
ASSISTANT PROFESSOR OF NEUROLOGY

John H. Morledge, M.D.
ASSISTANT RESIDENT IN MEDICINE

AND

Lowell W. Lapham, M.D.
ASSISTANT PROFESSOR OF PATHOLOGY

(From the Division of Neurology, Department of Medicine, Western Reserve University School of Medicine, Cleveland, Ohio)

Although hemorrhagic cerebral infarction occurs occasionally in response to thrombosis of cerebral vessels, in most instances in humans it has been found to result from cerebral embolus; thus it has been demonstrated that cerebral infarcts resulting from emboli are at least partially hemorrhagic in approximately 65% of cases (Adams and Vander Eecken[1]). Since there is considerable clinical data to support the widespread use of long-term anticoagulant therapy to reduce the hazard of further embolization in patients subject to recurrent embolic phenomena (Cosgriff[3] and McDevitt, Wright and Luckey[9]), the effect of anticoagulant drugs on the character of the cerebral lesions assumes considerable importance. Decision as to the influence of anticoagulant agents on the nature of cerebral infarcts in man, from study of pathological material alone, is difficult due to the great variation in the extent of hemorrhagic changes in infarcted areas in untreated cases, and because of difficulty in distinguishing spontaneous hemorrhage, especially in hypertensive individuals, from the effects of drugs. Judgment as to possible harmful effects of anticoagulant therapy solely from clinical data is likewise difficult, since deterioration of the clinical status of patients so treated may result from many other factors such as cerebral edema, multiple brain lesions, spontaneous cerebral hemorrhage, changing cardiac status, or other extracerebral intercurrent disease. Wright and McDevitt[14] reported 3 instances of apparent bleeding of the cerebral lesions in a group of 57 patients with thromboembolic brain disease treated with anticoagulant drugs; however, it was not clear how many of these 57 patients had suffered recent embolic infarction of the brain.

Since no well-documented control and treated series of cases has been reported, there has been some division of opinion among clinicians as to the safety of anticoagulant drugs in patients with encephalomalacia, especially when of recent origin, and especially when secondary to embolus. Thus the safety of anticoagulant agents in the treatment of patients with recent cerebral infarction has been questioned in

*Supported by Grant B–1281 from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

two recent symposia dealing with the problems of cerebrovascular disease (Symposium[13] and Wright and McDevitt[9,14]). On the other hand, Blumgart and co-workers[2] demonstrated no increased bleeding in *myocardial* infarcts experimentally produced in dogs with Dicumarol therapy. Reasoning from this, and because of data suggesting that infarcts in other organs in man are not apparently rendered more hemorrhagic by anticoagulation therapy, Wright speculated that such treatment might not be harmful in hemorrhagic cerebral encephalomalacia, although he emphasized that further study of this problem was necessary (McDevitt, Wright and Luckey[9], Wright and McDevitt[14]).

Thus there is no satisfying clinical data, and no experimental evidence, to allay the fears of the clinician that continuation or institution of anticoagulant therapy may increase bleeding in a recent area of brain infarction. Further, if one grants, on theoretical grounds, that such treatment may be potentially dangerous, the duration of the waiting period after which anticoagulants may be used with relative safety has not been clearly defined in cases in which such treatment is indicated for thromboembolic prophylaxis.

Since these problems are not clearly resolved by study of clinical experience and reports in humans, for reasons already stated, one of the first steps would seem to be to determine the effects of anticoagulant drugs on experimentally produced encephalomalacia in animals. Recently, Moyes and co-workers[10] have published the results of Dicumarol administration in a series of dogs in whom cerebral infarcts were produced by the injection of plastic vinyl acetate into the internal carotid artery; the brain lesions thus induced were significantly more hemorrhagic in anticoagulated animals than in a control series.

Methods. PRODUCTION OF INFARCTS. All methods which have thus far been used to produce brain infarcts in animals are subject to certain objections and limitations. We elected to use, with slight variation, a method of producing cerebral lesions in dogs by the injection of homologous clot fragments into the carotid artery, operatively exposed in the neck; the technique was described in detail by Hill, Millikan, Wakim and Sayre[8], and has the advantages of greater simplicity, higher incidence of infarction, and a relatively lower mortality rate, in comparison with methods previously used. Moreover, the employment of the animals' own clot would seem subject to less objection than the use of foreign substances to produce vascular obstruction.

Large healthy mongrel dogs, weighing 14 to 24 kg., were used in the present study. Five milliliters of blood were taken by venipuncture 48 hours before operation and allowed to stand at room temperature in a sterile test tube. Shortly before surgery, a portion of this clot was separated into 2 to 5 mm. fragments in a sterile Petri dish. These fragments were washed twice in sterile normal saline, and then transferred to a tuberculin syringe. Exactly 0.2 ml. of relatively compact fragments was then transferred to a 5 ml. syringe and re-suspended in 5 ml. of normal saline, and allowed to stand for periods of less than one hour prior to operation. Each animal received .0002 gm. of atropine sulfate hypodermically shortly before surgery. Anesthesia was induced with 5 to 10 ml. of a 2% Pentothal sodium solution intravenously and continued with drop ether, without intubation; cyanosis and respiratory difficulty were rarely encountered. Through a 4 to 6 cm. paratracheal incision, the common carotid artery and its branches were approached and identified. A temporary cotton ligature was then placed on the external carotid artery. The 0.2 ml. of clot fragments with the 5 ml. of isotonic saline solution in which they were suspended were then slowly injected through a 19 gauge needle into the common carotid artery; most of the clot fragments could be seen to pass into the internal carotid artery, although frequently some of them were seen to pass retrograde to disappear or to enter the thyroid artery; occasionally the course of the fragments could not be visualized. After injection the needle was withdrawn from the common caroti

artery, and bleeding from the puncture site was readily controlled in 2 to 3 minutes by manual nonocclusive compression at the point of puncture. The ligature was then removed from the external carotid artery, following which this vessel and its branches were always patent. The subcutaneous tissues and skin were then approximated with silk sutures.

SELECTION OF CASES, CONTROL AND TREATED SERIES. The procedure outlined above was performed in 50 dogs, and clinical signs of brain injury appeared in 36 of these animals. The first 18 animals showing clinical evidence of cerebral infarction were not anticoagulated, and served as controls. Since a previous study using this method of infarct production had shown that animals without neurological deficit almost invariably had no evidence of a brain lesion at pathological examination (Hill *et al.*[8]), and because our first few experiments confirmed this fact, animals having no clinical signs of brain damage were not sacrificed and were excluded from the study.

DICUMAROL ADMINISTRATION AND DEGREE OF ANTICOAGULATION MAINTAINED. In preliminary studies, postoperative bleeding occurred in the neck wound so commonly in heparin-treated subjects that the use of this drug was abandoned. Rather, the last 18 animals showing neurological deficit were given a single oral dose of bishydroxycoumarin (Dicumarol) in amounts varying from 150 mg. to 200 mg., or approximately 10 mg. per kg. of body weight. The rationale for the initial large-dose technique of Dicumarol administration has been described in detail by Shapiro and Weiner[12]. Usually Dicumarol was given 24 hours prior to surgery, but in several instances the drug was administered for the first time 1 to 4 days after cerebral infarction was produced. The time of Dicumarol administration, control prothrombin times, and prothrombin times on consecutive days after operation are included in Tables 1, 3, and 5 for each animal. Prothrombin time measurements were performed daily by the Link-Shapiro modification of the Quick technique, employing a commercially available thromboplastin-calcium solution (Simplastin). The thromboplastin-calcium solution was kept in a frozen state between determinations and proved to be stable during the course of the measurements, as judged by frequent control determinations in untreated animals. The same aliquot of thromboplastin-calcium solution was used for pretreatment and posttreatment determinations in any given animal.

Pretreatment and control prothrombin times were 11 seconds (± 1 sec.) in all but 4 of the 18 animals in the treated series. Prothrombin determinations are recorded in seconds in Tables 1, 3, and 5; however, on a prothrombin activity curve (Fig. 6), a range of 38 sec. to 20 sec. corresponds to the optimal clinical range of 11 to 23% prothrombin activity, by the method used.*

Prothrombin time exceeded 38 seconds on a single day in one animal (Animal 1, Table 3 and Fig. 3), and exceeded this level on more than one day in 6 animals (Animals 4, 6, and 7, Table 3 and Fig. 3; Animals 3, 4, and 5, Table 5 and Fig. 5); in the remaining 11 dogs in the treated series, prothrombin times never exceeded 38 seconds.

RECORDING OF CLINICAL OBSERVATIONS. The clinical signs of infarction were unmistakable and, when they appeared, were always evident within 1 to 2 hours after the operative procedure was completed. In the present study the animals were classified into 3 groups according to the severity of the neurological deficit:

Severe Signs. These animals remained stuporous 2 to 3 hours or longer after the operative procedure, and were never able to stand due to marked contralateral hemiparesis; there was marked forced turning of the head and eyes towards the side of the brain lesion, and varying degrees of restlessness and hyperventilation; they refused all fluids and nourishment. Almost all animals in this category died within 24 hours after operation.

Moderate Signs. Animals in this group became ambulatory within a few hours after surgery, but demonstrated persistent circling toward the side of the brain lesion, and did not spontaneously turn to the opposite side. Various degrees of contralateral hemiparesis were evident. These animals usually accepted fluids and food within 24 to 48 hours. Clinical signs persisted for longer than 3 days, although progressive improvement occurred in all animals in the control series.

Minimal or Transient Signs. These subjects showed a similar initial clinical picture but were completely free of gross neurological deficit by the end of 3 days, sometimes within 24 hours.

PATHOLOGICAL STUDIES. Survivors in both

*Although the Link-Shapiro method yields results which differ slightly from the Quick method when either normal or unusually low prothrombin ranges are measured, the two methods give closely approximate values in the range of 10 to 23% (Fisher[5]).

the control and treated series were sacrificed at various times, indicated in Tables 3 and 4, by intravenous injection of 10 ml. of a 2% Pentothal sodium solution, followed immediately by 10 ml. of intravenous ether. No animal was killed until significant clinical improvement had become evident, and in most instances neurological deficit had either not been evident, or had been unchanging, for several days prior to sacrifice. Brains were removed and suspended in 10% formalin solution for at least one week; the neck wound, chest and abdominal organs were inspected in all animals who had shown clinical evidence of extracerebral bleeding.

The brains were presented to the neuropathologist (L.W.L.), who was not informed in advance which specimens represented animals in the treated series. The brains were sectioned into 6 equal coronal slices and described in detail grossly, with special emphasis on the size and extent of infarction and the presence or absence of hemorrhagic manifestations. Since the gross appearance of the lesions was considered to be of greatest importance, microscopic sections were taken only in certain cases in which the extent of infarction was not clear from gross inspection.

Color photographs were made of all of the coronal sections in each case for study and permanent record. Single representative coronal sections from each animal were assembled in groups and are all reproduced in this report (Figs. 1, 2, 3, 4 and 5), with the exception of a single animal (see text below). It should be emphasized that each coronal section in the Figures represents a different animal, and in each instance is the section demonstrating the maximal extent of the infarction in that animal; sections revealing the maximal coronal extent of the infarct almost invariably show the maximal extent of the hemorrhagic portion of the infarct as well.

For descriptive purposes, infarcts measuring less than 15 mm. in greatest diameter were classified as small; large lesions were arbitrarily defined as those involving more than half of one hemisphere, and moderate sized infarcts were those measuring between these two extremes. Infarcts having no hemorrhagic changes, or only a few scattered petechiae, were classified as anemic in type; the remaining lesions were categorized as moderately or markedly hemorrhagic, and the accuracy of these estimations may be judged reasonably well by the reader from the photographs (Figs. 1, 2, 3, 4 and 5) and corresponding Tables.

Results. As might be expected, there was a rough correlation between the size of the cerebral lesions and the severity of clinical signs (see Tables). The size of the infarcts produced were comparable in both control and Dicumarol-treated animals. In both series there was a striking tendency for the larger lesions also to be the most hemorrhagic; however, there were noteworthy exceptions. Thus 2 animals in the control series (Animals 1 and 4, Fig. 4) and one animal in the anticoagulated series (Animal 7, Fig. 3) had small infarcts, which were, however, intensely or moderately hemorrhagic.

There was sufficiently marked variability in the severity and extent of hemorrhagic changes in the brains of both control and treated animals so that it was difficult to judge, with any accuracy, from study of pathological material alone, whether or not, in any given case, the animal had received Dicumarol. However, when the results of pathological studies were considered in correlation with the clinical observations, definite conclusions could be drawn.

EARLY DEATHS: CONTROL AND TREATED SERIES. Six deaths occurred in less than 24 hours in both the control and treated series of animals (Tables 1 and 2; Figs. 1 and 2). Almost all of these animals had severe symptoms after operation, and almost all had large infarcts in the brain; it thus seems most plausible to attribute death solely to the size of the cerebral lesions. Because the degree of hemorrhagic change in infarcted areas was approximately the same in both the treated and untreated groups, we regard the evidence as inconclusive that Dicumarol influenced the fatal outcome in the treated animals in this group of early deaths.

SURVIVORS: CONTROL AND TREATED SERIES. Eleven animals in the control

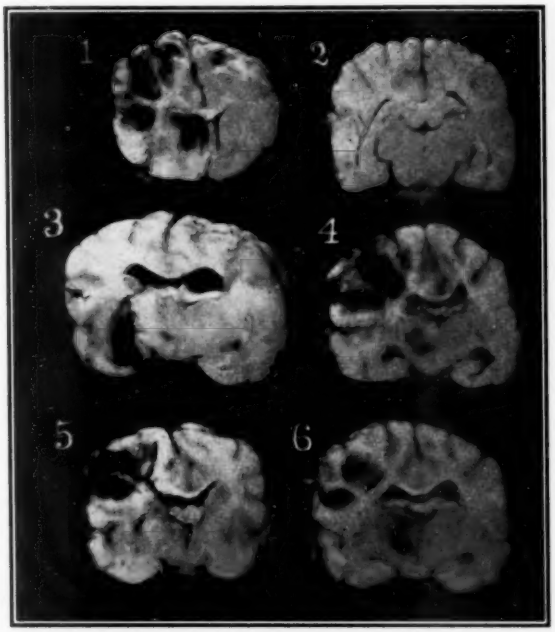

Fig. 1.—Representative coronal sections from the brains of the 6 animals surviving cerebral infarction for less than 24 hours and treated with Dicumarol. (See text and Table 1.)

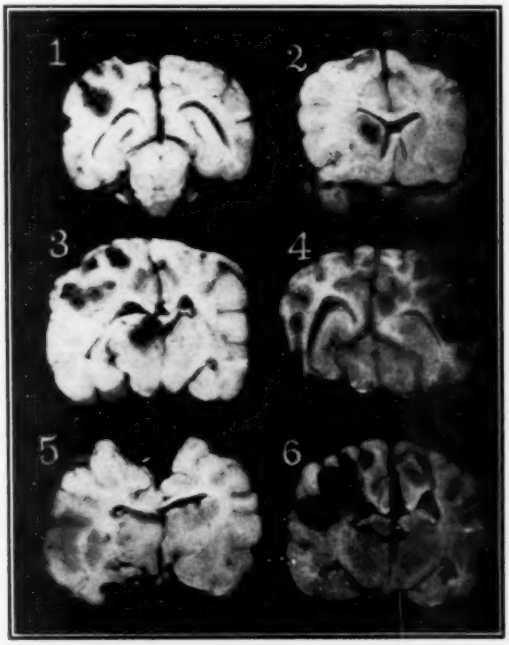

Fig. 2.—Representative coronal sections from the brains of the 6 animals in the control series who survived cerebral infarction for less than 24 hours. (See text and Table 2.)

group and 7 animals in the Dicumarol-treated series survived and were sacrificed 4 days to 6 weeks after the brain lesions were produced (Tables 3 and 4 and Figs. 3 and 4). The clinical course of all animals in both groups was characterized by progressive improvement and no animal showed worsening of neurological deficit once improvement became apparent.

It can be seen that in the control survivors' brains the areas of infarction were moderately or severely hemorrhagic in 8 of the 11 animals, while 5

TABLE 1.—DICUMAROL-TREATED ANIMALS SURVIVING INFARCTION LESS THAN 24 HOURS

*Dog No.**	*Severity Initial Symptoms*	*Size of Infarct*	*Extent of Hemorrhagic Changes in Infarct*	*Time Dicumarol Administered*	*Control Prothrombin Times (±1 sec.)*	*Prothrombin Time Day of Operation†*	*Time of Death (P.O.)*
1	Moderate	Large	Moderate	24 hrs. pre-op.	10 sec.	14 sec.	12 to 24 hrs.
2	Minimal	Small	Anemic	24 hrs. pre-op.	11 sec.	14 sec.	12 to 24 hrs.
3	Moderate	Large	Moderate	24 hrs. pre-op.	9 sec.	13 sec.	12 to 24 hrs.
4	Severe	Large	Marked	24 hrs. pre-op.	10 sec.	20 sec.	7 hrs.
5	Severe	Large	Marked	24 hrs. pre-op.	10 sec.	12 sec. (17 sec., 10 hrs. P.O.)	12 to 24 hrs.
6	Moderate	Large	Moderate	24 hrs. pre-op.	11 sec.	21 sec.	12 to 24 hrs.

* Representative sections of these animals' brains are depicted in Fig. 1, by corresponding numbers.

† Due to short survival times, prothrombin times were not measured just prior to death; however, these were almost certainly significantly prolonged at time of death, 36 to 48 hours after Dicumarol administration, since they were uniformly prolonged in surviving animals after this interval.

of the 7 anticoagulated survivors had cerebral infarcts which were predominantly anemic in type (Figs. 3 and 4).

The fact that there were more survivors in the untreated than in the treated series is, perhaps, of little significance because of the small numbers involved. However, it seems clear from study of the 2 groups that hemorrhagic infarcts were frequently compatible with survival and progressive clinical improvement in the control series; it was less common to find hemorrhagic infarcts in the brains of Dicumarol-treated survivors. It should also be noted that average or even excessive prolongation of prothrombin times did not prejudice improvement and survival in the 5 animals with anemic brain infarcts (Table 3 and Fig. 3).

LATE DEATHS: CONTROL AND TREATED SERIES. Only one of the 18 animals in the control series died more than 24 hours after operation. This animal had

TABLE 2.—CONTROL ANIMALS SURVIVING INFARCTION

*Dog No.**	*Severity Initial Symptoms*	*Size of Infarct*	*Extent of Hemorrhagic Changes in Infarct*	*Time of Death*
1	Moderate	Moderate	Moderate	12 to 24 hrs.
2	Severe	Large	Moderate	12 to 24 hrs.
3	Severe	Large	Marked	12 to 24 hrs.
4	Severe	Large	Marked	12 to 24 hrs.
5	Severe	Large	Anemic	Less than 12 hrs.
6	Severe	Large	Marked	12 to 24 hrs.

* Representative sections of these animals' brains are depicted in Fig. 2, by corresponding number.

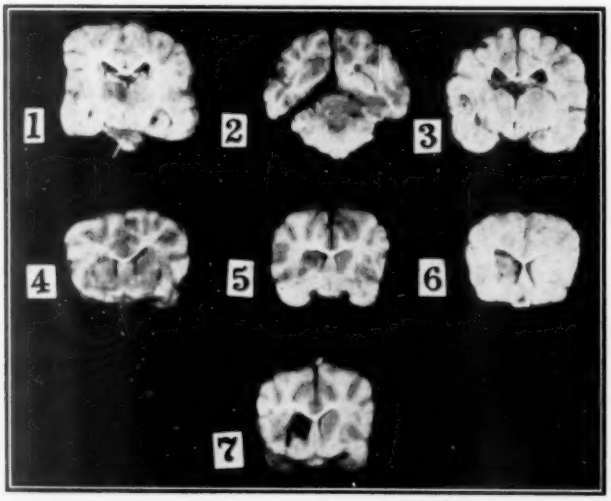

Fig. 3.—Representative coronal sections from the brains of the 7 animals in the Dicumarol-treated series who survived cerebral infarction. These animals were sacrificed at various times after the production of the cerebral lesions. (See text and Table 3.)

extremely severe clinical signs, and death occurred on the third postoperative day, with no improvement ever apparent prior to death; the cerebral infarct in this dog's brain involved almost an entire hemisphere and was intensely hemorrhagic; the brain fragmented excessively when sectioned, and this is the only animal in the entire study not represented photographically in this report. It is worth emphasizing that no animal in the entire control

TABLE 3.—DICUMAROL-TREATED ANIMALS SURVIVING INFARCTION

*Dog No.**	*Severity Initial Symptoms*	*Size of Infarct*	*Extent of Hemorrhagic Changes in Infarct*	*Time Dicumarol Administered*	*Control Prothrombin Times (±1 sec.)*	*Prothrombin Time Day of Operation and Successive P.O. Days†*	*P.O. Time Sacrificed*
1	Moderate	Small	Anemic	24 hrs. pre-op.	9 sec.	16, 18, 20, 30, 41	4 days
2	Minimal	Small	Anemic	24 hrs. pre-op.	11 sec.	19, 26, —, 32, 35, 30, 25, —, 20	6 weeks
3	Moderate	Moderate	Anemic	4 days post-op. and 6 days post-op.	9 sec.	8, —, —, —, 13, 24, 12, 17, 28, 24, 21, —, 15, —	10 days
4	Moderate	Small	Anemic	Day of op.	12 sec.	11, 14, 26, 38, —, 40, 45, 35, —, 24, 14, —	19 days
5	Moderate	Moderate	Moderate	3 days post-op.	11 sec.	11, —, —, —, 13, 26, 28, 32, 33, 33, 35, 34	11 days
6	Moderate	Small	Anemic	24 hrs. post-op.	13 sec.	13, —, —, 23, 39, 49	5 days
7	Minimal	Small	Marked	Day of op.	11 sec.	11, 18, 24, 39, 58, 45, 40, 36, —, 26, —, —, —, 12	14 days

* Representative sections of these animals' brains are depicted in Fig. 3, by corresponding numbers.

† A dash (—) means that no prothrombin time determination was done on that particular day.

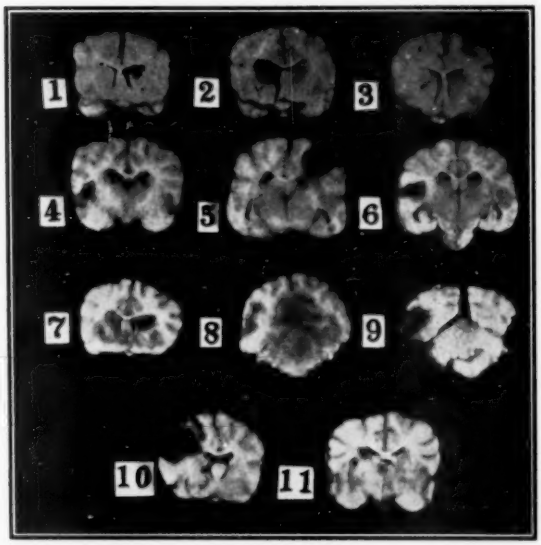

Fig. 4.—Representative coronal sections from the brains of the 11 animals in the control series who survived cerebral infarction, being sacrificed at various times indicated in Table 4. Note the higher proportion of hemorrhagic infarctions in these untreated survivors as compared to the survivors in the anticoagulated series. (Compare Fig. 3.)

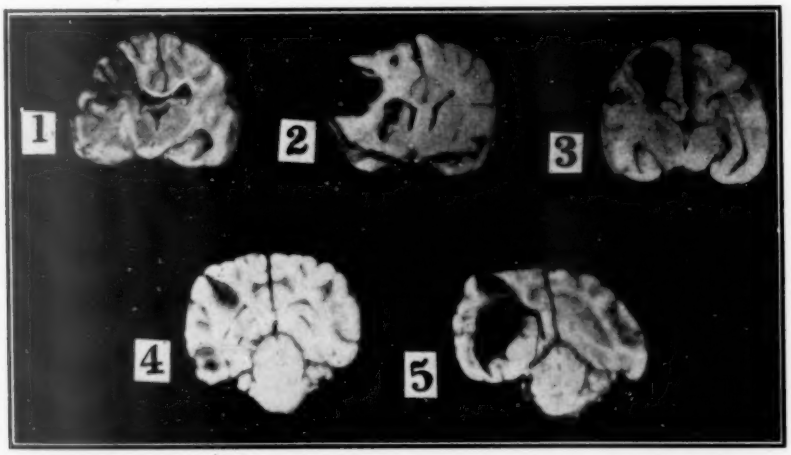

Fig. 5.—Representative coronal sections from the brains of the 5 animals with cerebral infarction, and treated with Dicumarol, who died several days after the infarcts were produced; Animals 1, 2, 3, and 4 died several days after infarction, after having shown initial moderate to marked clinical improvement, and death seemed clearly due to excessive hemorrhagic changes in the cerebral lesions in each instance. Animal 4, above, died primarily as a result of a large neck hematoma. (See text and Table 5.)

series ever showed clinical worsening after clinical improvement once became apparent.

By contrast, 4 of the 18 Dicumarol-treated animals died more than 24 hours after surgery, and death seemed quite clearly due to the cerebral lesion in each case (Animals 1, 2, 3, and 5, Fig. 5 and Table 5).

All of the 4 anticoagulated animals dying as a result of the brain lesions had a clinical course characterized by moderate to marked clinical improvement followed by gradual or sudden increase in neurological deficit and death; such a progression of events was never seen in the control series, and is, indeed, foreign to established clinical concepts of the natural history of a single cerebral infarct. One of these animals (No. 1, Fig. 5 and Table 5) had moderate initial signs of brain damage, but considerable improvement was apparent on the third day, after which time there was gradual increase in deficit, progressively increasing obtundation of consciousness, with death occurring approximately 96 hours after operation; the maximal prolongation of prothrombin time in this case was 30 seconds (control, 11 sec.), and the brain had a markedly hemorrhagic, moderately large infarct, with more extensive surface hemorrhage than in any of the control animals (Fig. 7 and No. 1, Fig. 5).

Another subject (Animal 2, Fig. 5) also showed definite clinical improvement and then died 4 days after operation, with a maximal prolongation of prothrombin time of 38 seconds (control, 12 sec.); the hemorrhagic infarct in this animal's brain was markedly firm on palpation in the fixed state, due to the large amount of blood in the infarcted area.

Animal 3 (Fig. 5 and Table 5) had moderate initial symptoms of brain injury, was strikingly improved in 4 days, and had no apparent neurological deficit on the sixth postoperative day when the prothrombin time was excessively prolonged (61 sec.); on the ninth day after infarction was pro-

TABLE 4.—CONTROL ANIMALS SURVIVING INFARCTION

*Dog No.**	*Severity Initial Symptoms*	*Size of Infarct*	*Extent of Hemorrhagic Changes in Infarct*	*Time of Sacrifice (Postop.)*
1	Minimal	Small	Moderate	4 days
2	Moderate	Moderate	Marked	4 days
3	Minimal	Small	Anemic	4 days
4	Minimal	Small	Marked	4 days
5	Moderate	Moderate	Marked	5 days
6	Moderate	Moderate	Moderate	5 days
7	Moderate	Small	Anemic	5 days
8	Moderate	Moderate	Anemic	5 days
9	Moderate	Moderate	Moderate	5 days
10	Severe	Large	Marked	4 days
11	Moderate	Moderate	Anemic	4 days

* Representative sections of these animals' brains are depicted in Fig. 4, by corresponding numbers.

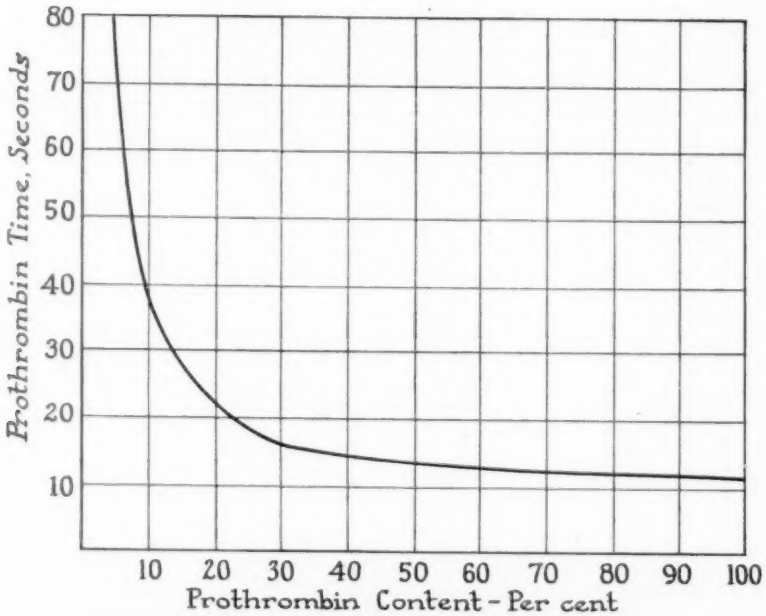

Fig. 6.—Relationship of the clotting time of the plasma to prothrombin content, calculated for control normal of 11 seconds (after Quick[11]).

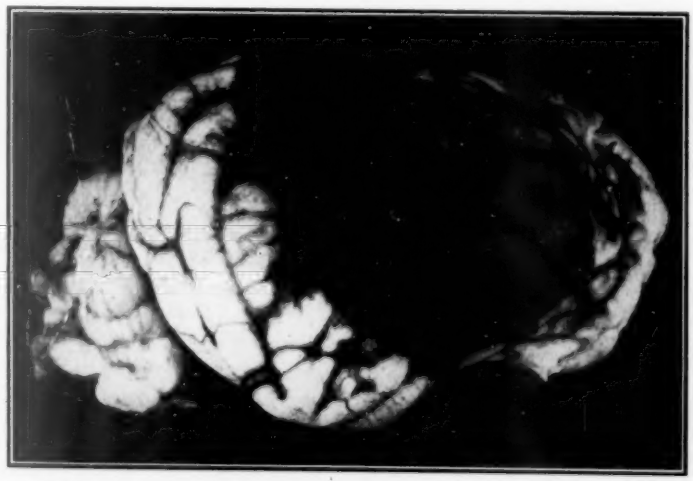

Fig. 7.—Large surface hemorrhage overlying a moderately large cerebral infarct in an animal treated with Dicumarol and dying on the fourth day after infarction, after showing improvement of clinical signs of brain damage on the third day. Maximal prolongation of prothrombin time was 30 seconds (control, 11 sec.). A coronal section of this animal's brain is shown in Fig. 5 (No. 1).

duced, with a similarly elevated prothrombin time, this animal suddenly became comatose and died; a moderately large cerebral lesion was found which was markedly hemorrhagic, and palpation of the infarcted area revealed it to be firm as in the previous case.

Animal 5 (Fig. 5 and Table 5) had moderate initial signs of brain injury which were markedly improved, but still evident, on the 12th day after operation, when the prothrombin time was prolonged to 42 seconds (control, 13 sec.), and declining from previous

TABLE 5.—DICUMAROL-TREATED ANIMALS DYING MORE THAN 24 HOURS AFTER INFARCTION*

Dog No.†	*Severity Initial Symptoms*	*Size of Infarct*	*Extent of Hemorrhagic Changes in Infarct*	*Time Dicumarol Administered*	*Control Prothrombin Times (±1 sec.)*	*Prothrombin Time Day of Operation and Successive P.O. Days*	*Time of Death (P.O.)*
1	Moderate	Large	Marked	24 hrs. pre-op.	11 sec.	13, 20, 27, 28, 30	4 days
2	Moderate	Large	Marked	24 hrs. pre-op.	12 sec.	13, 19, 22, 36, 38	4 days
3	Minimal	Moderate	Marked	24 hrs. pre-op.	12 sec.	21, 22, —, —, 33, —, 61, 64	7 days
4‡	Minimal	Small	Anemic	24 hrs. pre-op.	11 sec.	34, 40, 53	2 days
5	Moderate	Moderate	Marked	24 hrs. post-op.	13 sec.	13, 13, 17, 26, 30, 33, —, 55, —, 50, —, 42, —, —	14 days

* Only one animal in the control series died later than 24 hours after infarction (see text).

† Representative sections of these animals' brains are depicted in Fig. 5, by corresponding numbers.

‡ This animal did not die chiefly as a result of the brain lesion, but rather as the consequence of a large neck hematoma with anterior mediastinal extension; none of the other animals in this group had significant bleeding at the operative site.

higher levels; death occurred suddenly on the 14th day after infarction. This animal's brain, at pathological examination, had a moderate sized, markedly hemorrhagic infarct; at the posterior extremity of this infarct was a frank hematoma beneath the ependymal lining of the ventricular wall (No. 5, Fig. 5).

Discussion. The results of this study indicate that hemorrhagic cerebral infarcts in dogs may be made significantly more hemorrhagic by the administration of Dicumarol. There is little reason to believe that hemorrhagic infarcts in the human brain should behave differently under the influence of anticoagulant agents, although it has been difficult to conclusively settle this issue in human cases, for reasons previously mentioned. This tendency of hemorrhagic brain infarcts to bleed in subjects treated with anticoagulant drugs is in contrast to the apparent failure of these drugs to increase the hemorrhagic tendencies of infarcts in other organs.

Since there was approximately the same number of anemic infarcts in both the control and Dicumarol-treated series of animals (5 and 7, respectively), this investigation does not lend support to the hypothesis that originally anemic infarcts may be rendered hemorrhagic by treatment with anticoagulant drugs. The occurrence of significant hemorrhagic changes in brain infarcts is related chiefly to intermittent occlusion of arterial channels, usually due to a migratory embolus; the pathological evidence for this has been thoroughly studied and outlined by Adams and co-workers[1,6]. Experimentally, at least, other factors which accentuate the bleeding in an originally hemorrhagic cerebral softening are drug-induced hypertension (Globus *et al.*[7]), and increased cerebral venous pressure (Fazio and Sacchi[4]).

The implications of the present study with regard to the treatment of human patients with embolic infarction of the brain deserve comment. It would seem that anticoagulant agents should not be employed in the treatment of patients who have suffered recent cerebral infarction due to embolism. The length of time after hemorrhagic brain infarction when the use of these drugs may be considered safe was not thoroughly explored in this investigation; however, as already noted, in one instance sudden hemorrhage occurred in a 2-week-old hemorrhagic infarct, albeit in an animal with an excessively prolonged prothrombin time.

A clinical situation occasionally arises in which a patient already being treated with anticoagulant drugs suffers a cerebral embolus. In such an instance it would seem wise to return the clotting and prothrombin times to normal levels as soon as possible. The earliest time after cerebral infarction that hemorrhagic changes may develop has not been determined, but it seems probable that they might occur at any time during the first few hours. One animal in the present study (Animal 4, Table 1 and Fig. 1) had marked hemorrhagic changes in the brain lesion 7 hours after embolic arterial occlusion, and most of the animals in both treated and control groups that died between 12 and 24 hours after operation had markedly hemorrhagic cerebral infarcts.

Since infarcts resulting from thrombosis of cerebral arteries are usually, but not always, of the anemic variety, the results of this study would at first seem to have few implications relative to the use of anticoagulant drugs in cases of cerebral thrombosis. As mentioned previously, it seems unlikely from our data that originally anemic infarcts ever became hemorrhagic as a result of Dicumarol administration, al-

though this possibility cannot be completely dismissed. However, clinicians employing anticoagulant drugs in the treatment of cerebral thrombosis should recall that there is data revealing that the pathological diagnosis of brain embolism is made much more frequently than the clinical diagnosis of this condition; thus Adams and Vander Eecken[1] emphasize that the clinical diagnosis of cerebral thrombosis often is made erroneously in cases subsequently shown to be due to embolus. This difficulty in clinical differentiation of thrombosis and embolus apparently arises partly from the fact that the classical sources for cerebral embolus are not always present. It is probable that emboli arising from the walls of the aorta, carotid arteries, and pulmonary veins are more common than formerly appreciated (Adams and Vander Eecken[1]).

Summary. 1. Cerebral infarction was experimentally produced in 36 dogs by the injection of homologous clot fragments into the carotid artery in the neck; 18 of these animals served as controls, and 18 were treated with Dicumarol.

2. Grossly hemorrhagic cerebral infarction was frequently compatible with survival and progressive clinical improvement in the untreated series; thus, among 11 untreated animals surviving infarction, 8 had hemorrhagic cerebral infarcts.

3. A clinical course of progressive improvement was fatally reversed, as a result of hemorrhagic changes occurring in and about the cerebral lesions, in 4 of 12 animals surviving cerebral infarction by more than 24 hours, and treated with Dicumarol. In 2 such instances, death occurred in association with prolongation of prothrombin times of a degree not unusual in clinical practice. With greater prolongation of prothrombin times, death occurred as late as the 9th and 14th days after infarction in the 2 other cases.

4. Five of 7 animals surviving cerebral infarction and treatment with Dicumarol had brain infarcts which were grossly of the anemic variety.

Conclusion. The results of this study indicate that administration of Dicumarol increases the hemorrhagic tendencies of experimentally produced embolic brain infarcts in dogs. These findings are interpreted as providing confirmatory evidence for the belief of many clinicians that anticoagulant drugs are contraindicated after recent embolic cerebral infarction in humans. Caution is suggested in the use of anticoagulant agents in cases diagnosed clinically as cerebral thrombosis, because of difficulties in clinical distinction between thrombotic and embolic infarction of the brain.

REFERENCES

1. **Adams, R. D.,** and **Vander Eecken, H. M.:** Ann. Rev. Med., **4,** 213, 1953.
2. **Blumgart, H. L., Freedberg, A. S., Zoll, P. M., Lewis, H. D.,** and **Wessler, S.:** Am. Heart J., **36,** 13, 1948.
3. **Cosgriff, S. W.:** J. Am. Med. Assn., **143,** 870, 1950.
4. **Fazio, C.,** and **Sacchi, U.:** J. Neuropath. & Exper. Neurol., **13,** 476, 1954.
5. **Fisher, B.:** Am. J. Med. Sci., **215,** 39, 1948.
6. **Fisher, M.,** and **Adams, R. D.:** J. Neuropath. & Exper. Neurol., **10,** 92, 1951.
7. **Globus, J. H., Epstein, J. A., Green, M. A.,** and **Marks, M.:** J. Neuropath. & Exper. Neurol., **8,** 113, 1949.
8. **Hill, N. C., Millikan, C. H., Wakim, K. G.,** and **Sayre, G. P.:** Proc. Staff Meet., Mayo Clin., **30,** 625, 1955.

9. **McDevitt, E., Wright, I. S.,** and **Luckey, E. H.:** Cerebral Vascular Diseases. New York: Grune & Stratton, Inc., pp. 145–156, 1955.
10. **Moyes, P. D., Millikan, C. H., Wakim, K. G., Sayre, G. P.,** and **Whisnant, J. P.:** Proc. Staff Meet., Mayo Clin., **32**, 124, 1957.
11. **Quick, A. J.:** Am. J. Clin. Path., **10**, 222, 1940.
12. **Shapiro, S.,** and **Weiner, M.:** Am. Heart. J., **41**, 749, 1951.
13. Symposium of Inquiry. Cerebrovascular Disease: Neurology, **6**, 580, 1956.
14. **Wright, I. S.,** and **McDevitt, E.:** Ann. Int. Med., **41**, 688, 1954.

SUMMARIO IN INTERLINGUA

Infarcimento Cerebral Experimental: Le Effecto de Dicumarol

Le resultatos de iste studio indica que le administration de Dicumarol augmenta le tendentias hemorrhagic de embolic infarcimentos cerebral experimentalmente producite in canes. Iste constatation es interpretate como confirmation del opinion de multe clinicos que drogas anticoagulante es contraindicate post recente embolic infarcimentos cerebral in humanos. Es recommendate circumspection in le uso de agentes anticoagulante in casos diagnosticate clinicamente como casos de thrombosis cerebral a causa del difficultate de distinguer clinicamente inter thrombotic e embolic infarcimentos cerebral.

SUMMARIO IN INTERLINGUA

(See page 656 for original article)

Le Partition, Per Electrophorese A Papiro, De Proteinas Seral E Proteina C-Reactive In Patientes Con Bronchiectasis, Con Un Reporto Preliminari In Re Le Effecto De Prolongate Therapia Antibiotic

1. Varie determinationes de proteina seral esseva effectuate in 29 patientes con bronchiectasis.

2. Patientes con bronchiectasis monstrava depressiones significative del valores medie pro albumina e elevationes inconclusive del valores medie pro le fractiones de globulina alpha-2 e globulina gamma.

3. Proteina C-reactive esseva presente in le seros de quasi omne le patiente a un tempore o un altere.

4. Le comparation del configuration electrophoretic e del proteina C-reactive ante e post le tractamento non revelava ulle alteration de grado significative.

5. Es presentate le casos de 4 patientes additional con hypogammaglobulinemia e recurrente morbo pulmonar, incluse 2 con bronchiectasis. A parte le quasi complete absentia de globulina gamma, tres de iste patientes habeva valores elevate del globulinas alpha-1 e alpha-2 e de proteina C-reactive.

CLOTTING FACTOR ASSAYS ON PLASMA FROM PATIENTS RECEIVING INTRAMUSCULAR OF SUBCUTANEOUS HEPARIN*

By Samuel I. Rapaport, M.D.
ASSOCIATE PROFESSOR OF MEDICINE IN RESIDENCE
UNIVERSITY OF CALIFORNIA MEDICAL CENTER
LOS ANGELES, CALIFORNIA

AND

Sara Beth Ames, B.S.
SENIOR MEDICAL TECHNICIAN, DEPARTMENT OF MEDICINE
UNIVERSITY OF CALIFORNIA MEDICAL CENTER
LOS ANGELES, CALIFORNIA

(From the Department of Medicine, University of California Medical Center, Los Angeles, California, and the Medical Service, Veterans Administration Hospital, Long Beach, California)

This paper describes the behavior of plasma from patients given intramuscular or subcutaneous concentrated aqueous heparin in various clotting factor assays. Such information is needed when a patient who has received heparin bleeds, and one must quickly decide if the heparin, an unrelated coagulation defect, or an unsuspected local lesion is responsible. Conversely, patients with repeated thromboses may be suspected of hyperactivity of a clotting factor(s). These patients often receive heparin continuously and cannot be evaluated without knowing how this alters clotting factor assays.

Intravenous heparin prolongs the Quick "prothrombin time." The effect of intramuscular or subcutaneous heparin is not well documented. Yet, many physicians rely upon Quick "prothrombin times" when beginning coumarin anticoagulants in patients receiving heparin by these routes. Therefore, more information is needed on the reliability of the Quick "prothrombin time" in such patients.

Fear of hemorrhage still prompts physicians to obtain frequent Lee-White clotting times when giving heparin. An hour may be spent doing a single Lee-White test on blood containing heparin. Therefore, we hoped to find a rapid plasma clotting factor assay that would reflect heparin effect equally well.

Materials and Methods. REAGENTS. *Plasma containing heparin.* Blood was taken from patients with acute myocardial infarction receiving 100 mg. of heparin (Heparin sodium, Upjohn, 100 mg. per ml.) every 8 hours. The heparin was given either into the gluteal muscles or into the subcutaneous fat above the posterior iliac crest. Often, a patient was bled 2 to 4 hours after an injection and again just before the next injection. Nine parts of blood were added to 1 part of 2% sodium oxalate solution. Platelet-rich plasma was obtained by centrifuging at about 800 r.p.m., platelet-poor plasma by centrifuging at 4,000 r.p.m.

Saline extract brain thromboplastin. A saline-buffer suspension of human brain was prepared by Owren's technique[9] and stored liquid frozen in small aliquots at −20° C.

Crude "cephalin" suspension. An acetone insoluble, ether extract of human brain was made as previously described (Rapaport,

*These studies were supported in part by a grant from the Los Angeles County Heart Association and in part by grant #2453 from the National Heart Institute, United States Public Health Service.

Aas and Owren[11]). Small aliquots of a 2.2 gm. % lipid suspension in buffer were stored at −20° C., and diluted 1/50 in buffer before use.

Russell's viper venom "cephalin" reagent. Stypven (Burroughs-Wellcome Company, Inc.) was dissolved in dilute "cephalin" suspension as described elsewhere (Hjort, Rapaport and Owren[3]) and stored liquid frozen in small aliquots at −20° C. Activity diminished slightly over several weeks, following which the frozen preparation remained stable.

Adsorbed ox plasma. Oxalated ox plasma was adsorbed first by Seitz filtration and then by mixing with barium sulfate as described previously (Hjort, Rapaport and Owren[3]). This plasma, free of prothrombin and proconvertin but containing large amounts of proaccelerin and fibrinogen, was stored at −20° C. in aliquots suitable for one day's use.

Proaccelerin deficient human plasma. Oxygen was bubbled through approximately 250 ml. of oxalated human plasma until its pH rose to 8.2 units. The plasma was then incubated at 37° C. under oxygen for about 36 hours. Following this, carbon dioxide was bubbled through the plasma to correct its pH to about 7.0 units. This plasma contained adequate prothrombin, proconvertin and fibrinogen but only traces of proaccelerin activity. It was stored in small aliquots at −20° C.

Bovine thrombin. A stock thrombin solution, containing 100 units per ml. in 50% glycerol, was made from Parke Davis Topical Bovine Thrombin. One vial's contents were dissolved in 5 ml. of oxalated saline (1 part 2% sodium oxalate and 5 parts isotonic saline) and adsorbed with 100 mg. of barium sulfate for 20 minutes at 37° C. The barium sulfate was removed by centrifugation and 20 ml. of saline, followed by 25 ml. of glycerol were added to the supernatent. This preparation retained its potency stored in a glass bottle at 4° C. for many weeks.

A 1/10 dilution of this stock solution in buffer was consistently found to clot normal plasma in 20 to 25 seconds. The dilution was made with "silicone" technique to prevent adsorption of thrombin onto glass. The diluted thrombin was stable for 20 minutes in a silicone coated tube at 37° C.

Diluting fluid (Rapaport, Aas and Owren[12]) and *sodium diethyl barbiturate (veronal) buffer* (Rapaport, Aas and Owren[11]) were prepared as previously described.

METHODS. *Lee-White clotting time.* Blood was drawn into an untreated syringe. A stopwatch was started when blood first appeared in the syringe. One milliliter of blood was placed in each of three 10 by 75 mm. glass tubes, that were then placed in a 37° C. constant-temperature block. Tube 1 was tilted each minute until its contents could be inverted without spilling. Then, Tube 2 was tilted in the same manner, and, after it clotted, Tube 3. In 12 control subjects the longest clotting time found in Tube 1 was 10 minutes, in Tube 3, 16 minutes.

Partial thromboplastin time. In this test, devised by Langdell, Wagner and Brinkhous[5], a reagent supplying the equivalent of platelet thromboplastic activity is added to plasma that is then recalcified. We added 0.2 ml. of crude "cephalin" suspension diluted 1/50 in buffer to 0.2 ml. of the patient's plasma, and, after 3 minutes incubation at 37° C., 0.2 ml. of 30 mM, calcium chloride. Normal plasma clotted in 50 to 90 seconds.

Quick "prothrombin time." To 0.2 ml. of the patient's plasma were added, 0.2 ml. of human brain thromboplastin, and, after 3 minutes, 0.2 ml. of 20 mM calcium chloride. The thromboplastin used in these studies clotted normal plasma in 13.5 seconds.

Prothrombin-Proconvertin (P. and P.) test. This test, described by Owren and Aas[10], differs from the Quick test in that the patient's plasma is diluted, and adsorbed ox plasma is added to supply an optimal amount of proaccelerin and fibrinogen. Thus, to 0.2 ml. of the patient's plasma diluted 1/10 in diluting fluid, were added 0.2 ml. of adsorbed ox plasma and 0.2 ml. of human brain thromboplastin. After 3 minutes at 37° C., 0.2 ml. of 20 mM calcium chloride was added and the clotting time noted. The thromboplastin used in these studies gave a 33 second time with normal plasma.

Venom-"cephalin" prothrombin assay. This test, in which Russell's viper venom in "cephalin" suspension replaces brain thromboplastin, has been described in detail elsewhere (Hjort, Rapaport and Owren[3]). To 0.2 ml. of the patient's plasma diluted 1/40 in diluting fluid, were added 0.2 ml. of venom-"cephalin" reagent, and 0.2 ml. of adsorbed ox plasma. After 3 minutes at 37° C., 0.2 ml. of 37.5 mM calcium chloride was added and the clotting time noted. Normal plasma clotted in about 28 seconds.

Plasma thrombin time. A dilute thrombin solution was prepared as described above just prior to use. Small tubes containing 0.4 ml. samples of platelet-rich and of platelet-poor

plasma were placed at 37° C. together with the tube containing the dilute thrombin solution. After 3 minutes, 0.2 ml. of thrombin was added to the plasma and the clotting time noted.

Results and Discussion. 1. LEE-WHITE CLOTTING TIMES. Many Lee-White values accumulated as a by-product of this study. Their review brought out the following points:

a. A lag is expected between the first intramuscular or subcutaneous injection and therapeutic prolongation of the Lee-White time. To prevent this, 50 mg. of intravenous heparin are recommended with the first intramuscular dose. However, we found that the lag period could extend beyond the second intramuscular or subcutaneous injection. Thus, as Table 1 shows, if a third

TABLE 1.—CLOTTING TIMES AFTER THE SECOND HEPARIN INJECTION

	Third Tube Clotting Times (min.)			
	13–19	*20–24*	*25–30*	*>30*
No. Pts.	3	6	6	3

tube time of 25 minutes is arbitrarily considered a minimum therapeutic effect, 9 of 18 patients studied 2 to 4 hours after their second injection had yet to achieve it. Since impaired circulation following a myocardial infarction could delay absorption, these results may not apply to other types of patients. Nevertheless, if any patient is seriously threatened by thromboembolism, it would seem wise to check for heparin effect after the second intramuscular or subcutaneous injection, and, if unsatisfactory, to give more heparin intravenously.

Ten patients were followed through the second day of heparin therapy. Therapeutic prolongation of the Lee-White time was found in each on the second day. Thus, although somewhat slower than expected, a dosage schedule of 100 mg. every 8 hours did result in effective heparinization.

b. Lee-White clotting times were obtained on the third or fourth days of heparin, or both, in 5 patients receiving intramuscular and 6 patients receiving subcutaneous injections. Usually, the Lee-White times were longer on these days than on the second day. Moreover, in 4 of 6 patients on subcutaneous heparin, the times were longer than necessary. For example, a 152-pound man with congestive failure exhibited third tube clotting times just *before* the afternoon heparin injection of 20 minutes on the first, 38 minutes on the second, and more than 60 minutes on the third day of therapy.

In 11 patients, clotting times were obtained after stopping heparin. As the examples in Table 2 illustrate, some

TABLE 2.—CLOTTING TIMES AFTER STOPPING HEPARIN

Pt.	*Days on Hep.*	*Route*	*Hrs. after Hep.*	*Clot. time (3rd tube) (norm. <17 min.)*
R.B.	5	im	10	25
			13	15
S.M.	6	sub	10	30
			13	30
			17	23
J.B.	7	im	10	25
			13	26
			17	12
R.C.	10	im	16	37

heparin effect persisted from about 11 to over 17 hours after the last injection. This should be remembered when undertaking such procedures as lumbar puncture, sympathetic block or thoracentesis shortly after discontinuing heparin. These data imply that if heparin is required for more than 3 or 4 days, that is, beyond the time required for prothrombin depression by coumarin anticoagulant, a maintenance dosage of 100 mg. every 12 hours should suffice.

2. THE INSENSITIVITY OF THE PARTIAL THROMBOPLASTIN TIME TO HEPARINEMIA. In the partial thromboplastin time test, "cephalin," a reagent with platelet thromboplastic factor-like activity, is added to the patient's plasma. This makes the clotting time dependent only upon the plasma clotting factors. The test is more sensitive than the Lee-White clotting time in detecting "mild" hemophilia. Our experience is that normal plasma will clot in 50 to 90 seconds, plasma from a severe hemophiliac in 150 to 1,000 seconds, and plasma from a "mild" hemophiliac, who may have a normal Lee-White clotting time, in 100 to 150 seconds.

In contrast, the partial thromboplastin time proved less sensitive than the Lee-White time in detecting heparinemia. Blood containing enough heparin to double the Lee-White time almost always exhibited a normal partial thromboplastin time. When Lee-White times were prolonged 3- or 4-fold, the partial thromboplastin time sometimes exceeded 100 seconds, but, as Table 3 illustrates, often remained normal.

TABLE 3.—FAILURE OF THE PARTIAL THROMBOPLASTIN TIME TO REFLECT HEPARINEMIA

Pt.	*Clot. Time (3rd tube) (norm. <17 min.)*	*Part. Thrombopl. Time (norm. 50–90 sec.)*
L.C.	10	52
	>30	55
J.M.	15	65
	>52	74
H.L.	10	52
	>60	72

"Cephalin" can neither neutralize heparin's antithrombin properties (Wolf[16] and Rapaport and Ames[13]), nor prevent heparin's interference with prothrombin conversion (Rapaport and Ames[13]). Since both of these actions of heparin occur at therapeutic levels (MacMillan and Brown[7] and Douglas[2]), the reason why the partial thromboplastin time is insensitive to heparin is unknown.

3. THE UNPREDICTABILITY OF THE QUICK "PROTHROMBIN TIME" AFTER INTRAMUSCULAR OR SUBCUTANEOUS HEPARIN. Either the Quick or the Prothrombin-Proconvertin (P. and P.) test can be used to guide coumarin anticoagulant therapy. One of the latter method's advantages is that dilution of the patient's plasma eliminates error due to a circulating anticoagulant such as heparin.

The Quick "prothrombin time" lengthens after intravenous heparin and even has been used to follow such therapy (Olwin[8]). Despite this, we were not certain how the plasma levels obtained after intramuscular or subcutaneous injection would affect the test. Therefore, Quick and P. and P. test results were compared in 11 patients, 4 of whom also received a coumarin anticoagulant.

As expected, we found that heparin did not influence the P. and P. test. In contrast, fluctuating Quick "prothrombin times" were observed in 7 patients. The data from 4 are summarized in Table 4. The first 2 patients received only heparin, the last 2, as the drop in P. and P. values suggests, a coumarin anticoagulant as well.

These data illustrate the unpredictability of the Quick test. Thus, H.L. had "prothrombin" values of 15% and 64%, two and a half and eight hours respectively, after an injection of heparin, despite a Lee-White time of more than 60 minutes on both occasions. In the last 2 patients, values obtained 8 hours after an injection, that is, just prior to the next injection, were still much too low.

The Quick test remained accurate in 4 studies despite long Lee-White times. The reason is unknown but possibly

related to brain thromboplastin's antiheparin properties (Brambel, Corwin and Capone[1]). Our thromboplastin was made in a single batch and stored liquid frozen in small aliquots. Although thromboplastic potency did not vary from vial to vial, it is conceivable that antiheparin activity did. In any event, until more is known, the Quick test using human brain thromboplastin must be considered unreliable for regulating coumarin anticoagulant therapy in patients receiving intramuscular or subcutaneous heparin.

When the technique was first described (Hjort, Rapaport and Owren[3]), it was shown that adding 100 mcg. of heparin to each ml. of anticoagulant (as is done in the Ware-Stragnell modification[15] of the P. and P. test) prolonged the clotting time. This occurred despite the initial 1/40 dilution of the patient's plasma that is made in the test. Therefore, it seemed possible that therapeutic heparin levels might alter the test. However, as the data on 3 patients listed in Table 5 illustrate, this did not happen. The venom-"cephalin"

TABLE 4.—QUICK AND P. AND P. TEST RESULTS AFTER HEPARIN

Pt.	*Days on Hep.*	*Hrs. since last inj.*	*Clot. time (3rd tube) (min.)*	*Quick test %*	*P. and P. Test %*
J.B.	1	8	16	90	100
	2	2½	>60	70	100
	3	2	70	37	100
		8	39	51	100
H.L.	0	0	10	60	86
	1	2½	>37	47	94
		8	45	44	92
	2	2½	>60	15	98
		8	>60	64	86
J.M.	0	0	15	74	87
	1	2½	34	60	75
		8	23	45	70
	2	1½	>50	14	45
		8	36	<10	39
E.H.	8	2	>33	<10	34
		8	28	<10	35
		19	18	47	42

4. THE PROTHROMBIN ASSAY WITH VENOM-"CEPHALIN" IN HEPARINEMIA. This test uses Russell's viper venom dissolved in a "cephalin" suspension in place of brain thromboplastin. It was originally thought to be specific for prothrombin but may also reflect variation in Stuart factor (Hougie[4]). It must not be used to regulate coumarin anticoagulant therapy for it is uninfluenced by proconvertin. However, this insensitivity to proconvertin enhances its value for prothrombin consumption studies, and it has replaced the P. and P. test for this purpose in our laboratory.

TABLE 5.—RESULTS OF THE VENOM-"CEPHALIN" PROTHROMBIN ASSAY ON PLASMA CONTAINING HEPARIN

Pt.	*Clot. Time (3rd tube) (min.)*	*Ven.-"Ceph." Proth. Time (%)*
L.C.	10	+100
	>30	+100
E.W.	13	67
	39	71
	29	73
H.G.	19	100
	35	100

assay was uninfluenced by intramuscular or subcutaneous heparin injection and may be used with confidence when patients receive heparin by these routes.

5. A ONE-STAGE PROACCELERIN ASSAY OF PLASMA CONTAINING HEPARIN. Proaccelerin (plasma Ac. globulin) activity diminishes rapidly when heparin is added to plasma *in vitro* (Lewis and Ware[6] and Brambel, Corwin and Capone[1]). This led Brambel and his co-workers[1] to postulate that proaccelerin inactivation plays a key role in heparin's anticoagulant action. However, when proaccelerin was measured in plasma from patients on heparin, no inactivation could be demonstrated. Table 6 shows the stability of proaccelerin values despite varying Lee-White clotting times.

TABLE 6.—RESULTS OF A PROACCELERIN ASSAY ON PLASMA CONTAINING HEPARIN

Pt.	*Clot. Time (3rd tube) (min.)*	*Proacc. Level (%)*
A.K.	28	100
	40	100
H.G.	19	100
	35	100
G.T.	21	55
	60	62
J.M.	42	100
	60	88

This does not necessarily mean that heparin exerts its therapeutic effect independently of proaccelerin inactivation. The 1/20 initial dilution made in our assay could mask inactivation present in undiluted plasma. However, if so, dilution readily reactivates proaccelerin.

These data clearly demonstrate that the results of a proaccelerin assay employing diluted plasma are valid in patients receiving therapeutic amounts of heparin. Moreover, we have recently found our assay accurate on dogs' blood rendered completely incoagulable by heparin.

6. THE THROMBIN TIME OF PLASMA CONTAINING HEPARIN. Heparin, a potent antithrombin, markedly altered plasma thrombin time tests. Dilute thrombin that would clot normal plasma in 20 to 25 seconds, failed to clot plasma from blood with a third tube Lee-White time over 30 minutes. When the Lee-White time was less than 25 minutes, platelet-rich and platelet-poor plasma often behaved differently. As Table 7 shows, the former usually clotted readily whereas the latter did not.

TABLE 7.—THE THROMBIN TIME OF PLATELET-RICH AND PLATELET-POOR PLASMA CONTAINING SMALL AMOUNTS OF HEPARIN

	Clot. Time (3rd tube)	*Thrombin Time (sec.)*	
Pt.	*(min.)*	*Plat.-rich*	*Plat.-poor*
E.W.	14	37	no clot
E.J.	19	35	no clot
C.	22	37	no clot

This difference reflects platelet's known ability to neutralize heparin (Van Creveld and Paulssen[14]). Presumably this neutralization involves binding of heparin to platelets. If so, this bond must break during centrifugation. Otherwise a heparin-platelet complex would sediment and platelet-rich and platelet-poor samples would behave alike.

Table 8 summarizes the results of 50 thrombin time tests on platelet-poor and platelet-rich plasma and relates them to the Lee-White clotting time. These data illustrate that the thrombin time of platelet-poor plasma is a sensitive test for heparin. Note that this test sometimes detected trace heparinemia insufficient to prolong the Lee-White time. Although 5 of 10 platelet-poor thrombin times were still below 50 seconds when the Lee-White time was between 17 and 20 minutes, all 26 determinations were grossly abnormal on plasma from blood with a

Lee-White time longer than 20 minutes. Therefore, we have adopted the thrombin time of platelet-poor plasma as a screening test for heparinemia.

The thrombin time of platelet-rich plasma, less sensitive to small amounts of heparin, proved a better indicator of therapeutic prolongation of the Lee-White time. Thus, clotting failed to occur in 18 of 21 determinations when the Lee-White time was more than 25 minutes, but in only 3 of 30 determinations when the Lee-White time was less than 25 minutes.

whose settings for platelet-rich and platelet-poor samples have been predetermined. Stability of the dilute thrombin can be insured by making the dilution in silicone treated glassware and by using the dilute material within 20 minutes. If these simple precautions are observed the test should prove reliable for routine clinical use.

Summary and Conclusions. Concentrated aqueous heparin given intramuscularly or subcutaneously in a dosage of 100 mg. every 8 hours affected various clotting assays as follows:

TABLE 8.—RELATION OF LEE-WHITE TIME TO THROMBIN TIME OF PLATELET-RICH AND PLATELET-POOR PLASMA

Clot. time (3rd tube) (min.)	*Thrombin time (20–25 sec. norm.)*					
	Platelet rich (sec.)			*Platelet poor (sec.)*		
	<50	*50–300*	*no clot*	*<50*	*50–300*	*no clot*
<17	*11	3	1	9	2	4
17–20	7	2	1	5	0	5
20–24	3	1	1	0	1	4
25–30	1	2	10	0	1	12
30	0	0	8	0	0	8

* The numbers in these columns are the number of determinations that fell within the indicated clotting ranges.

These data indicate that a properly standardized plasma thrombin time test can replace the Lee-White clotting time in following heparin therapy. If the purpose is to rule out residual heparinemia, platelet poor plasma should be used, if to check for a therapeutically effective heparin level, platelet-rich plasma. The advantage of the test is its great saving of time. Instead of being tied to the bedside for many minutes as in a Lee-White test, a technician may take blood from several patients, return to the laboratory and complete the tests in rapid succession.

The potential sources of error are in preparing the plasma samples and the dilute thrombin. The former can be eliminated by using a centrifuge

1. Lee-White clotting times were prolonged to therapeutic levels in every patient. However, the time required to reach a therapeutic level often extended beyond the second injection. Elevated Lee-White times occasionally persisted for more than 17 hours after heparin was stopped.

2. The partial thromboplastin time proved a poor indicator of heparinemia. Even when Lee-White times were prolonged 3- or 4-fold, normal or only slightly prolonged partial thromboplastin times were obtained. Therefore, a long partial thromboplastin time in a patient receiving heparin should suggest the possibilities of either extreme overdosage or the existence of a second coagulation defect.

3. The Quick "prothrombin time"

test using human brain thromboplastin fluctuates unpredictably. Tests on blood taken just before the next injection were sometimes in error. Therefore, the Quick test should not be used to regulate coumarin anticoagulant therapy in patients also receiving intramuscular or subcutaneous heparin.

4. The Prothrombin-Proconvertin (P. and P.) test, the specific prothrombin assay with Russell's viper venom in "cephalin" suspension, and a one-stage proaccelerin assay were all uninfluenced by the heparin levels resulting from this route of administration. These tests give valid results in such patients.

5. The plasma thrombin time was markedly altered by heparinemia. When the Lee-White time was less than 25 minutes, platelet-rich plasma often clotted readily whereas platelet-poor plasma did not. This difference can be explained by assuming that a heparin-platelet complex breaks when platelets are sedimented by centrifugation.

The thrombin time of platelet-poor plasma proved a sensitive indicator of, and is recommended as a simple screening test for, heparinemia. The thrombin time of platelet-rich plasma could be related to therapeutic prolongation of the Lee-White time. Our data suggests that a properly standardized plasma thrombin time can replace the Lee-White time in following heparin therapy.

ACKNOWLEDGMENT: We wish to thank Dr. L. E. Sansum for helping select some of the patients for these studies.

REFERENCES

1. **Brambel, C. E., Corwin, A. H.,** and **Capone, V. A.:** Am. J. Med. Sci., **230,** 276, 1955.
2. **Douglas, A. S.:** J. Clin. Invest., **35,** 533, 1956.
3. **Hjort, P., Rapaport, S. I.,** and **Owren, P. A.:** J. Lab. & Clin. Med., **46,** 89, 1955.
4. **Hougie, C.:** Proc. Soc. Exper. Biol. & Med., **93,** 570, 1956.
5. **Langdell R. D., Wagner, R. H.,** and **Brinkhous, K. M.:** J. Lab. & Clin. Med., **41,** 637, 1953.
6. **Lewis, M. L.,** and **Ware, A. G.:** Proc. Soc. Exper. Biol. & Med., **84,** 640, 1953.
7. **MacMillan, R. L.,** and **Brown, K. W. G.:** J. Lab. & Clin. Med., **44,** 378, 1954.
8. **Olwin, J. H.:** Surg., Gynec. & Obst., **90,** 423, 1950.
9. **Owren, P. A.:** Scandinav. J. Clin. & Lab. Invest., **1,** 81, 1949.
10. **Owren, P. A.,** and **Aas, K.:** Ibid., **3,** 201, 1951.
11. **Rapaport, S. I. Aas, K.,** and **Owren, P. A.:** J. Lab. & Clin. Med., **44,** 364, 1954.
12. Iidem: J. Clin. Invest., **34,** 9, 1955.
13. **Rapaport, S. I.,** and **Ames, S. B.:** Proc. Soc. Exper. Biol. & Med., **95,** 158, 1957.
14. **Van Creveld, S.,** and **Paulssen, M. M. P.:** Lancet, **2,** 242, 1951.
15. **Ware, A. G.,** and **Stragnell, R.:** Am. J. Clin. Path., **22,** 791, 1952.
16. **Wolf, P.:** Brit. J. Hematol., **2,** 375, 1956.

SUMMARIO IN INTERLINGUA

Essayage De Factor Coagulatori In Plasma Ab Patientes Recipiente Heparina Per Via Intramuscular O Subcutanee

Le administration intramuscular o subcutanee de concentrate heparina aquose in doses de 100 mg omne 8 horas afficeva varie essayos de coagulation in le sequente maniera:

1. Le tempores de coagulation secundo Lee-White esseva prolongate a valores

therapeutic in omne casos. Tamen, le intervallo ante is attingimento del valores therapeutic durava frequentemente usque ultra le secunde injection. Prolongate tempores de Lee-White persisteva in certe casos durante plus que 17 horas post le ultime uso de heparina.

2. Le tempore de thromboplastina partial se provava pauco efficace como indicator de heparinemia. Mesmo in casos in que le tempores de Lee-White esseva triplicate o quadruplicate, le tempores de thromboplastina partial se monstrava normal o non plus que levemente prolongate. Per consequente, prolongation del tempore de thromboplastina partial in un patiente tractate con heparina deberea suggerer le possibilitate de un extreme excesso de dosage o del existentia de un secunde defecto coagulatori.

3. Le test del "tempore prothrombinic" secundo Quick con le uso de thromboplastina ab le cerebro human monstrava fluctuationes sin ulle predictibilitate. Tests facite con specimens de sanguine que habeva essite obtenite immediatemente ante le proxime injection produceva a vices resultatos erronee. Ergo, le test de Quick non deberea usar se pro regular therapias anticoagulante a coumarina in patientes qui etiam recipe heparina intramuscular o subcutanee.

4. Le test a prothrombina-proconvertina (cognoscite como P e P), le essayo de prothrombina specific con veneno viperin de Russell in un suspension de "cephalina," e un essayo de proaccelerina a phase unic, omne istos non esseva influentiate per le nivellos de heparina que resulta ab le via de administration usate. Iste tests produce resultatos valide in tal patientes.

5. Le tempore de thrombina del plasma esseva marcatemente alterate per heparinemia. Quando le tempore de Lee-White esseva minus que 25 minutas, il occurreva frequentemente que plasma ric in plachettas se coagulava prestemente, durante que le contrario valeva pro plasma povre in plachettas. Iste differentia pote esser explicate per supponer que un complexo de heparina e plachettas es disrumpite quando le plachettas es sedimentate per centrifugation.

Le tempore de thrombina in plasma a basse contento de plachettas se provava un sensibile indicator de heparinemia. Iste test es recommendate como simple manovra de "detection" pro heparinemia. Le tempore de thrombina in plasma ric in plachettas poteva esser correlationate al prolongation therapeutic del tempore de Lee-White. Nostre datos suggere que un appropriatemente standardisate tempore de thrombina de plasma pote prender le placia del tempore de Lee-White in le observation del curso de therapia a heparina.

THE DEFINITIVE DIAGNOSIS OF EFFUSIVE OR CONSTRICTIVE PERICARDITIS*

By Louis A. Soloff, M.D.
PROFESSOR OF CLINICAL MEDICINE AND HEAD OF THE DIVISION OF CARDIOLOGY, TEMPLE UNIVERSITY MEDICAL SCHOOL AND HOSPITAL

AND

Jacob Zatuchni, M.D.
ASSISTANT PROFESSOR OF MEDICINE, TEMPLE UNIVERSITY MEDICAL SCHOOL AND HOSPITAL

(From Temple University School of Medicine and Hospital, Philadelphia, Pennsylvania)

Effusive or constrictive pericardial disease may be extremely difficult or impossible to diagnose by conventional means in the absence of characteristic calcification which itself is not pathognomonic of constriction (Harvey *et al.*[1]). This is so because the pericardium cannot be isolated from the cardiac silhouette by conventional roentgenologic methods and also because all of the cardiovascular derangements produced by pericardial disease, including hemodynamic findings uncovered by the technique of cardiac catheterization, may be mimicked by primary myocardial or endocardial disturbances produced by a variety of diseases. For this reason, we turned first to angiocardiography and later to intravenous carbon dioxide, following its introduction by Durant *et al.* and proof of its safety in the human[2].

Angiocardiography is informative and is capable of uncovering pericardial effusion, (which had previously been reported by Williams and Steinberg[13]), and also constrictive pericarditis. This type of study permits a simple differentiation of the two. Equally important, it allows for analysis of the relative contributions of myocardial and pericardial factors in the production of cardiac disability. Pericardial effusion and constrictive pericarditis may also be recognized by carbon dioxide contrast study which is simpler to do although it is not always as effective in identifying with certainty either the presence or nature of extraluminal change nor, of course, does it tell anything of the functional nature of the disturbance. Various aspects of this subject have been previously reported elsewhere and were alluded to by Durant *et al.*[2].

Our purpose is to summarize the diagnostic features of chronic pericardial disease as revealed by angiocardiography, a technique which also uncovers associated myocardial disease or dysfunction.

Case Reports. case 1. E.L., a 46-year-old woman, was admitted to Temple University Hospital on May 28, 1956, because of increasing dyspnea, fatigue, intermittent fever and weight loss of 8 years' duration. During this time, she was studied at various hospitals and was found to have progressive enlarge-

*Supported in part by a grant from the Heart Association of Southeastern Pennsylvania.
This paper formed part of lectures delivered before the American College of Chest Physicians, February, 1957; King's County Medical Society, March, 1957; and Osler Lecture, Medical School, University of Vermont, May, 1957.

ment of the heart and on two occasions, 4 and 2 years ago, positive blood cultures. Active rheumatic fever, bacterial endocarditis and thyroid disease were mentioned to her as possibilities but no definitive diagnosis was ever made.

Examination revealed a thin, slightly built woman who had a low grade fever. The thyroid gland was not palpable. The jugular veins were distended. The apex impulse, tapping in character, was in the sixth intercostal space at the left anterior axillary line. P2 was split, accentuated and louder than A2. A blowing diastolic murmur of moderate intensity was audible along the left upper parasternal region and a blowing systolic murmur of grade 2 intensity was heard along the left lower parasternal region and at the apex. The rhythm was regular at a rate of 60 per minute. The lungs were clear. The liver was enlarged and slightly tender. There was no edema. Radioactive iodine uptake was normal. Blood cultures were sterile.

Electrocardiogram showed complete heart block and diminished amplitude of the QRS complexes.

Chest roentgenograms revealed normal findings in 1948 and progressive enlargement of the cardiac silhouette since that time. The present roentgenograms were interpreted as showing "tremendous enlargement of the cardiac silhouette evidently involving all chambers on the basis of mixed valvular heart disease." The report continued, "The possibility of pericardial effusion contributing to the tremendous enlargement of the cardiac silhouette should be considered although no significant change in configuration is noted between a film made with the patient lying supine and a film made with the patient erect."

The nature of the underlying disorder was equally puzzling to the clinicians. Angiocardiography was done on June 20, 1956.

Extensive density, due to fluid, was found surrounding the heart and great vessels. The greatest amount of fluid was present on each side of the heart, particularly the right. A smaller amount was found inferior to the heart and the least amount directly anterior and posterior to the heart.

The superior vena cava was widened and slightly displaced to the right. There was reflux opacification of the inferior vena cava which was also dilated and wider than the superior vena cava. The inferior vena cava was directed rightward and slightly posterior from its position at the level of the diaphragm. The right atrium was also displaced to the right. Its inferior one-third was more posterior than usual. The right atrium appeared small in the anterior projection and large in the lateral projection, particularly inferiorly. The tricuspid valve region also appeared narrowed on the anterior projection and widened on the lateral projection. The right ventricle was compressed from side to side and from front to back but was otherwise normal. It varied in size during the cardiac cycle. The pulmonary vasculature appeared within the limits of normal other than superior displacement of the pulmonary artery, particularly its left branch. The left atrium was enlarged to 263 ml. The left ventricle was also enlarged. During diastole, the left atrium and left ventricle were homogeneously opacified. Representative angiocardiograms displaying these findings are shown in Fig. 1.

The duration of opacification of the superior vena cava was 7.0 seconds, of the right heart 8.4 seconds, of the pulmonary artery 9.1 seconds and of the left atrium 13.3 seconds. The intracardiac circulation time, that is, the time from the initial appearance of contrast substance in the right atrium to that in the ascending aorta, was 7.7 seconds. (Normally, the intracardiac circulation time is 6.3 seconds and is not exceeded by the duration of opacification of either the right heart or pulmonary artery or left atrium which are approximately equal.)

On the basis of these findings, a pericardial aspiration was done and several liters of serosanguineous fluid obtained. Subsequently, pericardiocenteses were done repeatedly with the removal of more fluid and consequent symptomatic relief. Corticoid therapy was begun and the patient was discharged.

She continued to feel well until corticoid dosage had to be reduced for economical reasons. Symptoms re-appeared and she was re-admitted to Temple University Hospital on April 5, 1957.

An angiocardiogram was done and once more pericardial effusion was demonstrated. Its amount was much less than before. The underlying cardiac structure and flow through it, as revealed by angiocardiography, were identical to that previously noted. Exploratory thoracotomy for inspection of the heart, biopsy of the pericardium and possibly open drainage were suggested but permission was not obtained. She was discharged again on corticoid therapy.

Angiocardiography clearly defined the nature of this problem.

The angiocardiographic diagnosis of pericardial effusion depends upon the

demonstration of homogeneous density surrounding the opacified heart. In the absence of a cardio-pericardiac fistula, contrast substance does not leave the cardiac chambers to enter the pericardial sac. Hence, the intrapericardial density, although opaque, is in marked contrast to the opacified heart. It is adjacent to all of the heart borders but is thickest on each side, particularly the right, to a lesser extent inferiorly and to the least degree directly anteriorly and posteriorly to the heart. The angle formed by the outer border

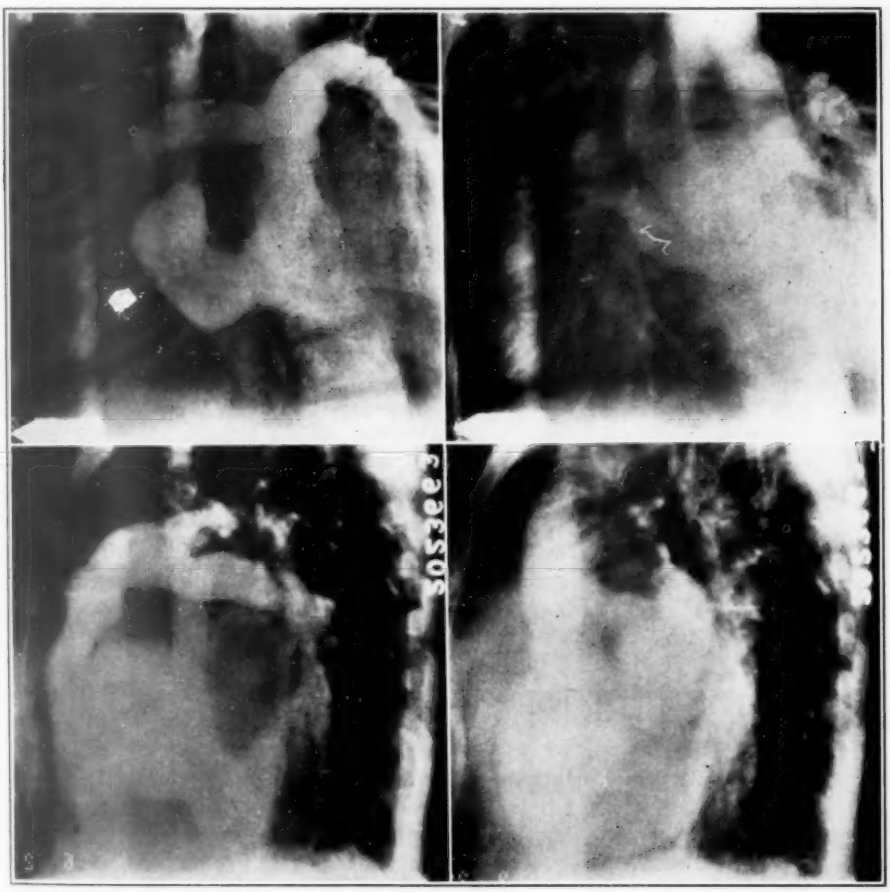

Fig. 1.—Case 1, E.L. (Pericardial Effusion). Simultaneous anterior (*upper*) and left lateral (*lower*) angiocardiograms at 2.8 seconds (*left*) and 9.8 seconds (*right*) after appearance of opacity in the superior vena cava. On the left, the superior vena cava, right atrium and ventricle, and pulmonary artery and branches, are opacified. On the right, the pulmonary veins, left atrium and ventricle and aorta are opacified. The opacified heart is surrounded by a uniform density. Note the slight rightward displacement of the superior vena cava and right atrium, and the superior displacement of the pulmonary artery, particularly its left branch. Extraluminal density adjacent to the left lateral border of the pulmonary artery is increased. Underlying mitral stenosis is reflected in the enlargement of the left atrium. The left ventricle is displaced forward. There is reflux opacification of the inferior vena cava. Both vena cavae are dilated.

of the pericardial density and the diaphragm is acute (Levene and Kaufman[7]). These characteristics are important to exclude lesions like neoplasm, cyst or abscess, which may also produce density extrinsic to the opacified heart (Robb[9]). Such density is characteristically asymmetrical or irregular in shape. If pericardial effusion is produced by an underlying neoplasm, localized change in density may occur.

The heart itself remains predominantly undeformed by the effusion and, in the absence of restriction by pericardial thickening, shows variation in size during the cardiac cycle. Its position also undergoes no material change, as was pointed out by Connor[1] in 1925, other than occasional but slight rightward displacement of the superior vena cava and right atrium.

The opacified cardiovascular structures may also reveal findings which occur secondary to the pericardial effusion. Thus, for example, the vena cavae are dilated and the dilatation of the inferior one is greater. The time of opacification of the superior vena cava is prolonged. The inferior vena cava is refluxly opacified by contrast substance from the right atrium, a finding which is unusual in normal individuals and rather frequent in those with increased right atrial pressure or tricuspid regurgitation. There was no other evidence to support the latter possibility, except for possible widening of the tricuspid region on the lateral projection.

The right ventricle, although compressed, maintains its general shape and varies, at least to some degree, during the cardiac cycle. The pulmonary artery and its branches, particularly the left, are displaced superiorly by the underlying effusion which also increases adjacent extraluminal density. The pulmonary veins may be prominent and the left atrium enlarged if there is interference with left ventricular filling. The left ventricle is displaced forward by the pericardial effusion. The forward displacement of the left ventricle and the relatively little fluid anterior to it may explain the presence, in a considerable proportion of cases, of a diffuse wavy cardiac impulse over the body of the heart and the not infrequent recognition of a distinct apex beat (Connor[1]). In the absence of left ventricular restriction, the left ventricle is not enlarged and shows variation in size during the cardiac cycle. At times, opacity may disappear during systole which suggests little residual blood.

Such findings permit easy recognition of the presence of pericardial effusion and its effects. Additional findings should incite suspicion of associated disease.

The occasional finding of almost normal intracardiac circulation times with massive pericardial effusion and the absence of change in times after removal of pericardial fluid suggest that increased intrapericardial pressure, sufficient to cause obvious distention of the jugular veins has a limited influence on the speed of flow of blood through the heart as revealed by angiocardiography. Characteristically, pericardial effusion is associated with a normal intracardiac circulation time and slightly prolonged duration of opacification of the superior vena cava or right heart. Prolonged intracardiac circulation time suggests myocardial dysfunction, most probably related to underlying intrinsic myocardial disease. Angiocardiography, which permits a study of the size, shape and content of the cardiac chambers and great vessels and the flow of contrast substance through them, makes it possible frequently to pinpoint the nature of the gross myocardial fault. Thus, in the

case just described, the angiocardiographic findings were characteristic of rheumatic heart disease with mitral stenosis (Soloff *et al.*[11]) surrounded by massive pericardial effusion.

If one is interested simply in proving the anatomic presence of pericardial effusion, 100% carbon dioxide may be injected intravenously with the patient in the left lateral decubitus position and the Roentgen-ray beam horizontal. Such a demonstration, for instance, was shown through the courtesy of Dr. Linton Turner who consulted us about a patient with myxedema heart. One of us, with Kern, Bello and Snape[6], had previously reported on the frequency of pericardial effusion in this syndrome. Dr. Turner consented to the procedure and the illustrations have been reported elsewhere (Paul *et al.*[8]).

The distance between the outer borders of the gas bubble and cardiac silhouette is increased. In this type of study, the demonstration in pericardial effusion of an increase in extraluminal thickness depends upon the presence of a sufficient amount of pericardial fluid remaining adjacent to the right atrial border with the patient in the left lateral decubitus position. Obviously, pericardial and myocardial thickening, as well as localized extrapericardial densities, must be excluded. It is also important to recognize that the superior vena cava, as well as the right atrium, may be illuminated by carbon dioxide and that these two contrast shadows may fuse to produce an illusion of a greatly enlarged right atrium. The simplicity, availability, usefulness and harmlessness of this type of study suggest its routine use to attempt to establish a diagnosis of pericardial effusion.

Angiocardiography and carbon dioxide contrast study may specifically diagnose constrictive pericarditis.

CASE 2. F.J., a 27-year-old colored man, was admitted to Temple University Hospital on December 1, 1956, because of dyspnea and ankle edema, appearing 6 years after the occurrence of hemoptysis and a right serosanguineous pleural effusion for which no cause was uncovered. Later, urine cultures were found to contain tubercle bacilli. During his present hospitalization, he had ankle edema, distention of the jugular veins and a right pleural effusion.

Electrocardiogram showed notched P waves, diminished amplitude of the QRS complexes and inverted T waves.

Chest roentgenogram showed enlargement of the cardiac silhouette, extensive pericardial calcification and a right pleural effusion.

Following intensive medical therapy, including digitalis and mercurial diuretics, cardiac catheterization disclosed findings characteristic of constrictive pericarditis.

Angiocardiography showed that the pericardial calcification was adjacent to the superior vena cava, right atrium, right ventricle and left ventricle. The superior vena cava was fixed in a dilated position. It did not vary in size from film to film. The inferior vena cava, refluxly opacified by contrast substance from the right atrium, was also dilated and larger than the superior vena cava. The lateral border of the right atrium was not convex. Rather, it was straight with a slight tendency to convexity inwards. The extraluminal density adjacent to the lateral border of the right atrium was 5 mm. thick. The right atrium was enlarged. The left lateral border of the right ventricular outflow tract was straight rather than convex inwards and did not vary from film to film. The pulmonary artery was enlarged and its branches were almost at the same level. The pulmonary veins were prominent. The left atrium was enlarged to 345 ml. Adjacent to the left ventricular cavity, extraluminal density was at least 2 cm. thick. Selected angiocardiograms are shown in Fig. 2.

The angiocardiographic circulation times were all greatly prolonged. The intracardiac circulation time was 10.5 seconds. The duration of opacification of the superior vena cava was 10.5 seconds, of the right heart at least 14.0 seconds, of the pulmonary artery at least 12.6 seconds and of the left atrium at least 9.8 seconds.

Carbon dioxide contrast study was also done and the roentgenogram has been published elsewhere (Paul *et al.*[8]).

Pericardiectomy was done on December 12, 1956. Dense plaques of calcium were found over both ventricles. The right atrial wall,

although grossly free of calcium, was thickened and fused with the adjacent pericardium. The pericardium over both ventricles was removed except for a small area on the left ventricle where a calcific plaque infiltrated the myocardium.

Postoperatively, he did well until the fifth day when he developed a mediastinal abscess, pneumonia and heart failure. Following intensification of cardiac therapy, mediastinal drainage and antibiotics, he gradually recovered.

of the superior vena cava, becomes straight and does not vary in contour during the cardiac cycle. Similar restriction may occasionally be seen affecting the superior vena cava or right ventricle, particularly in the region of the outflow tract. The left ventricle may also be similarly affected but overlapping density frequently obscures it. The effect of such restriction is seen

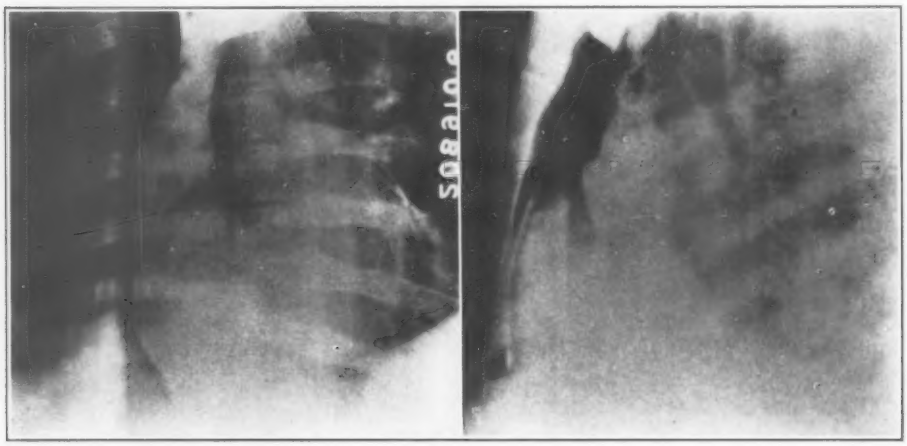

Fig. 2.—Case 2, F.J. (Constrictive Pericarditis). Simultaneous anterior (*left*) and left lateral (*right*) angiocardiograms at 3.5 seconds after appearance of opacity in the superior vena cava. Both vena cavae are dilated and the superior vena cava is rigid. Calcification is present adjacent to the anterior border of the superior vena cava, right atrium and ventricle. Calcification is also seen in the region of the left atrium and ventricle. The right lateral border of the right atrium is not convex. Rather, it is relatively straight with a tendency to slight convexity inwards. It did not change in succeeding films. Adjacent extraluminal density is 5 mm. thick. The right atrium is enlarged, particularly inferiorly. The left lateral border of the outflow tract of the right ventricle is straight and did not vary.

Angiocardiography and carbon dioxide contrast study permitted specific recognition of constrictive pericarditis. The pathognomonic finding is a rigid right atrial border which has lost its normal outward convexity. This feature of constrictive pericarditis has been independently described by Figley and Bagshaw[3]. No longer is there a continuous outward curve from the superior vena cava to the right atrium. Rather, the entire lateral border of the right atrium, beginning at the entrance in the dilated vena cava, pulmonary vessels and left atrium. The right atrium is also enlarged and its enlargement is most prominent inferiorly where it spreads posteriorly to receive the widened inferior vena cava.

The intracardiac circulation time is variable and depends upon associated myocardial dysfunction. In the case described above, the intracardiac circulation time was greatly prolonged, even following intensive cardiac therapy. In other instances, the intracardiac circu-

lation time shortens following such therapy or may be short even without therapy.

CASE 3, L.B., a 44-year-old colored man, was admitted to Temple University Hospital on December 7, 1956, because of substernal distress and slight dyspnea upon climbing a ladder. Examination revealed a well-developed muscular male. The jugular veins were not distended. There was systolic depression in the mid-precordial region followed by a diastolic rebound. The apex impulse was difficult to visualize because of overlapping density. Both atria showed adjacent pericardial calcification. The lateral border of the right atrium was straight, rigid and calcified (Fig. 3). Adjacent extraluminal density was variable. In some films, none was seen. In others, it was at most 5 mm. thick. The duration of opacification of the superior vena cava was 7.0 seconds, of the right heart 6.3 seconds, of the pulmonary artery 6.3 seconds and of the left atrium 15.4 seconds. The intracardiac circulation time was 6.3 seconds.

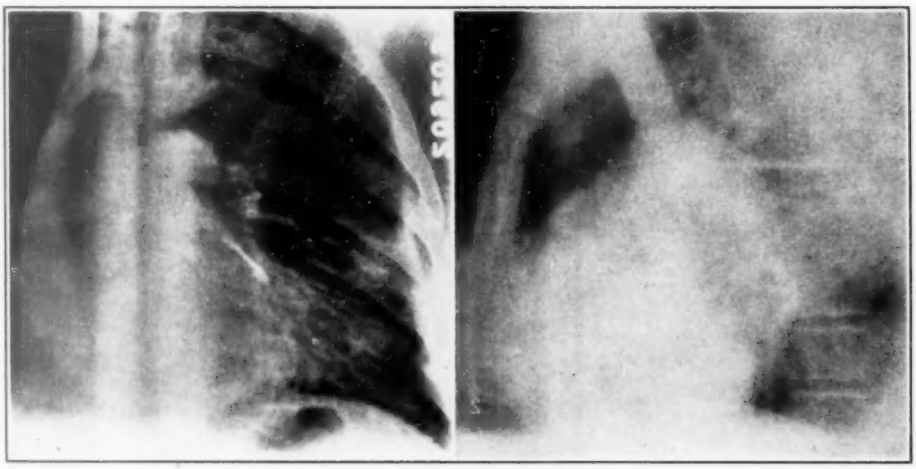

Fig. 3.—Case 3, I.B. (Constrictive Pericarditis). Simultaneous angiocardiograms in anterior (*left*) and left lateral (*right*) projections 1.4 seconds after appearance of opacity in the superior vena cava. The vena cavae are dilated. The right lateral border of the right atrium is straight and rigid. No extraluminal opacity is seen. The right atrium is enlarged, particularly inferiorly. Calcium is adjacent to the atria and ventricles.

was not visible. A2 was equal to P2. There were no murmurs. The blood pressure was 130/86 mm.Hg. The venous pressure was 160 mm. (citrated blood).

Electrocardiogram showed notched P waves and inverted T waves.

Chest roentgenogram showed a slightly enlarged cardiac silhouette with pericardial calcification.

Cardiac catheterization revealed findings characteristic of constrictive pericarditis.

Angiocardiography revealed a widened superior vena cava. The inferior vena cava, refluxly opacified by contrast substance from the right atrium, was also dilated. The right atrium, right ventricle, pulmonary vessels and left atrium were enlarged. The left ventricle

Pericardiectomy was done on December 15, 1956. There was very little calcium on the ventricles. The thickened pericardium was easily separated from the left ventricle and then from the right ventricle. The atria were embedded in calcific plaques which could not be removed. The anterior and circumflex coronary arteries were extensively calcified.

Biopsy showed a densely fibrous and hyalinized matrix containing blood vessels with thickened walls. There was patchy eosinophilic cellular infiltration adjacent to blood vessels.

The postoperative course was uneventful other than the transient appearance of heart failure.

On January 23, 1957, the angiocardiographic study was repeated and showed no significant change other than increased opacification likely related to the removal of thickened pericardium.

We have come to regard persistent prolongation of the intracardiac circulation time following adequate medical therapy as rather ominous, for the intracardiac circulation time has remained similarly prolonged following surgery. Although it might be said that if surgery was adequate, normal cardiac function should be restored, the fact remains that these patients, although improved by surgery, continue to have signs of cardiac dysfunction. It may be further stated that in our own experience these cases have been found at surgery to have plaques so intimately connected with the ventricular myocardium that their removal was impossible. The myocardial dysfunction, therefore, may be at least partly attributed to the limitation of surgery, but it may also be related to intrinsic myocardial disease either primary or secondary to prolonged pericardial restriction (White[12]). Intrinsic myocardial disease is also suggested by the finding of a rigid right atrial wall. Regardless of adjacent roentgenographically visible pericardial thickening, (which occasionally is not seen), the right atrial wall has remained rigid following surgery perhaps because of the inability to remove from it the fused pericardium. The rigidity of the right atrial wall may possibly have some relation to the persistence of abnormal physical signs following an otherwise adequate pericardiectomy, although experimentally in dogs with isolated pericardial constriction of the right atrium, no change in hemodynamics or physical signs occurred (Isaacs, Carter and Haller[5]) and, in the human, no abnormal fall in pressure between the superior vena cava and right atrium or between the right atrium and ventricle has been demonstrated (Sawyer *et al.*[10]). For these reasons, we would regard this sign as less significant from the prognostic viewpoint than a persistently prolonged intracardiac circulation time.

Finally, it must be emphasized again that we regard the finding of either a prolonged intracardiac circulation time following medical therapy or a rigid right atrial wall not as contraindications to surgery, for our patients have been improved by operation, but rather as indicative of myocardial dysfunction which may persist following surgery, either because of our present inability to decorticate the heart adequately or because of associated irreversible myocardial disease.

Summary and Conclusions. 1. Pericardial effusion or constrictive pericarditis may be definitively recognized by venous angiocardiography or carbon dioxide contrast study.

2. Extraluminal surrounding density due to fluid is the pathognomonic finding of pericardial effusion.

3. A rigid relatively straight right lateral border of the right atrium is the pathognomonic finding of constrictive pericarditis. Rigidity of the superior vena cava and of the right ventricle may also be demonstrated.

4. Either diagnosis is supported by the finding of dilatation of the venae cavae, predominantly the inferior.

5. Sequential venous angiocardiography permits recognition of underlying myocardial disease or dysfunction.

REFERENCES

1. Connor, L. A.: Am. Heart J., 1, 421, 1925.
2. Durant, T. M., Stauffer, H. M., Oppenheimer, M. J., and Paul R. E., Jr.: Ann. Int. Med., 47, 191, 1957.

3. **Figley, M. M.,** and **Bagshaw, M. D.:** Radiology, **69,** 46, 1957.
4. **Harvey, R. M., Ferrer, M. I., Cathcart, R. T., Richards, D. W.,** and **Cournand, A.:** Circulation, **8,** 695, 1953.
5. **Isaacs, J. P., Carter, B. N., II,** and **Haller, J. A., Jr.:** Bull. Johns Hopkins Hosp., **90,** 259, 1952.
6. **Kern, R. A., Soloff, L. A., Snape, W. J.,** and **Bello, C. T.:** Am. J. Med. Sci., **217,** 609, 1949.
7. **Levene, G.,** and **Kaufman, S. A.:** Radiology, **57,** 373, 1951.
8. **Paul, R. E., Jr., Durant, T. M., Oppenheimer, M. J.,** and **Stauffer, H. M.:** Am. J. Roentgenol., **78,** 224, 1957.
9. **Robb, G. P.:** An Atlas of Angiocardiography. Washington: American Registry of Radiology, 1951.
10. **Sawyer, C. G., Burwell, C. S., Dexter, L., Eppinger, E. O., Goodale, W. T., Gorlin, R., Harken, D. E.,** and **Haynes, F. W.:** Am. Heart J., **44,** 207, 1952.
11. **Soloff, L. A., Zatuchni, J., Stauffer, H. M.,** and **Kelly, E. W.:** Circulation, **13,** 334, 1956.
12. **White, P. D.:** Circulation, **4,** 288, 1951.
13. **Williams, R. G.,** and **Steinberg, I.:** Am. J. Roentgenol., **61,** 41, 1949.

SUMMARIO IN INTERLINGUA

Le Diagnose Definitive De Pericarditis Effusive O Constrictive

1. Effusion pericardial o pericarditis constrictive pote esser recognoscite definitivemente per angiocardiographia venose o studios a contrasto per bioxydo de carbon.

2. Densitate circumjacente extraluminal, causate per fluido, es le constatation pathognomonic de effusion pericardial.

3. Un rigide e relativemente directe margine dextero-lateral del atrio dextere es le constatation pathognomonic de pericarditis constrictive. Rigiditate del vena cave superior e del ventriculo dextere pote etiam esser demonstrate.

4. Ambe diagnoses es supportate per le constatation de dilatation del venas cave, predominantemente le vena cava inferior.

5. Angiocardiographia venose sequential permitte le recognition de un subjacente morbo o dysfunction myocardial.

DIFFERENCES IN PLASMA TOTAL BASE AND CATIONS IN SEVERE RENAL DISEASE*

By Makepeace U. Tsao, Ph.D.
ASSISTANT PROFESSOR OF BIOCHEMISTRY

AND

Jeana Levinthal, M.D.†
INSTRUCTOR OF PEDIATRICS

WITH THE ASSISTANCE OF

Lillian J. Wyngarden, A.B.

Charles H. Sloan, B.S.

AND

Shirley S. Sethna, B.S.

(From the Department of Pediatrics and Communicable Diseases, University of Michigan Medical School, Ann Arbor, Michigan)

An interesting and significant difference has been observed by us over the past 4 years between the plasma total base value as determined by electrodialysis and the sum of values of sodium, potassium and calcium of the plasma in several children and adults with severe renal disease. Although only the few patients reported here manifest such difference, a difference as high as 40 mEq. per liter has been observed. In view of the importance of the retention of nitrogenous substances in uremia which are normally excreted, an attempt to characterize and identify the unknown substance(s) causing such difference was made on the plasma extract, and the result is also presented in this report.

Materials and Methods. Venous or arterial blood samples were drawn with dry heparinized syringes as described previously (Reardon *et al.*[7]). Sixty samples were taken from a control group of pediatric patients with various diseases where water, electrolyte or acid-base disturbance was suspected. Twenty-one samples were taken from 13 adults and children who were uremic. The samples were chilled in ice water until analyses to prevent possible bacterial activity on nitrogenous material in the sample.

Blood urea nitrogen was determined by the alpha-isonitrosopropiophenone method (Archibald[1]).

Sodium and potassium were determined with a Beckmann Flame Spectrophotometer. The plasma sample was diluted with distilled water 1:100. A set of sodium standards was used for comparison which contained the normal amounts of potassium, calcium, magnesium and phosphorus. The equivalent concentrations were spaced 10 mEq. per L. apart. For each concentration of sodium standard a set of potassium standards was prepared with the equivalent concentration of potassium spaced 1 mEq. per L. apart. Each sample was read "bracketed" between two standards of closest sodium and potassium concentrations. A turbidimetric method was used for the determination of calcium (Murayama[4]).

*Aided by a grant from the Faculty Research-Research Equipment Fund of the Horace H. Rackham School of Graduate Studies, University of Michigan.

†Present address: Jimmy Fund Building, Binney Street, Boston, Mass.

The total base of the plasma was determined by an electrodialysis procedure (Sobel, Rockenmacher and Kramer[9], Tsao[11]). This method yields the sum of Na, K, and Ca, ammonia and some organic bases, but does not include Mg.

Paper chromatographic technique was used for the identification of organic bases in the plasma. It was assumed that whatever the substances might be they are extractable by the following procedure. The plasma extracts were prepared by dispersing 1 ml. of plasma into 5 ml. of 2% acetic acid in absolute alcohol, centrifuging after one hour, evaporation of 4 ml. of the supernatant to dryness with air stream, rinsing the residue twice with 5 ml. of chloroform, taking up the residue in 2 ml. of absolute alcohol, removal of insoluble residue by filtration, evaporation of the alcoholic solution to dryness, rinsing again with chloroform and finally dissolving the residue in 40 μl. of distilled water. For the chromatographic work 20 μl. of the extract were placed on Whatman No. 1 filter paper, dried and developed at room temperature with n-butanol saturated with 25% acetic acid in water (Schwyzer[8]). Organic bases are spotted by spraying with 1% alcoholic solution of bromphenol blue. A distinct blue spot shows the location of a nitrogenous base on the yellow background of the paper. Both ascending and descending techniques were employed. The following substances were used for comparison of R_f of the unknown: adenine, arginine, 1, 4-butanediamine, choline, citruline, creatine, creatinine, ethanolamine, guanidine, guanine, histidine, isoamylamine, lysine, ornithine, trimethylamine, and trimethylamine oxide.

Results. Twenty samples of the control group were analysed for Na, K, Ca, and total base. The sum of the cations was compared with the total base value of each sample. The results are summarized in Table 1. Since the calcium level fluctuates within a narrow range even in pathological conditions a constant value of 5 milliequivalents per liter (mEq./L.) was assumed. Forty samples were analysed for Na, K and total base and the results are also summarized in Table 1. From the data in the table it is obvious that the probability of differences of 6 mEq. per L. or more due to methodology is 0.05 or less.

In the group of patients in question, 6 children and 4 adults, differences of 10 to 40 mEq. between total base and the sum of Na, K, and Ca were observed on one or more occasions in the course of their illness (see Table 2). Their histories are given below.

TABLE 1.—COMPARISON OF TOTAL BASE WITH CATIONS IN CONTROL GROUP

Difference = d mEq./L.	*Total Base—* $(Na + K + Ca)$	*Total Base—* $(Na + K + 5)$
No. of samples = N	20	40
Range of d	$-4.0 \rightarrow +4.0$	$-6.0 \rightarrow +5.0$
Average d $= \Sigma d / N$	0.25	0.46
Average Abs. d $= \Sigma \lvert d \rvert / N$	2.2	2.8
$\sqrt{d^2/(N-1)}$	2.5	3.1

It should be noted that the blood urea nitrogen level does not bear direct relationship to the presence of significant amount of unknown substance(s) other than the fact that the levels are high in all of the uremic patients studied. Furthermore, the possibility of high concentrations of urea interfering with the determination of Na, K, and total base in these cases was ruled out by experiments involving the addition of urea to plasma samples up to the amount of 1000 mg. per 100 ml. without significantly alter-

ing the results either of electrodialysis or flame photometry. The case histories of 3 other patients, 3 adults with severe renal diseases and problems of uremia, who by contrast did not retain the unknown substance(s) are presented as examples of many uremic patients who did not manifest a difference between total base and known cations.

TABLE 2.—COMPARISON OF TOTAL BASE WITH SODIUM AND POTASSIUM IN UREMIA

Patients	*Blood Urea Nitrogen (BUN) mg./100 ml.*	*Na mEq./L.*	*K mEq./L.*	*Sum of Na, K, Ca*	** Total Base mEq./L.*	*Total Base mEq./L. From Unknown Substance(s)*
Case 1. R. B.	207	136	6.1	147	167	20
	500	142	6.6	154	187.5	34
	—	146	6.8	158	198	40
	—	145	8.0	158	188	30
	850	137	7.9	150	151.5	2
Case 2. D. B.	—	127	7.3	139	150	11
	210	127	7.7	140	159	19
	245	131	6.7	143	163	20
Case 3. N. R.	124	133	7.9	146	160.5	15
Case 4. R. J.	392	140	4.7	150	162	12
Case 5. P. DeW.	—	130	8.6	144	160	16
Case 6. L. W.	261	138	5.1	148	160	12
	218	139	5	149	153	4
	48	144	4.5	154	153	−1
Case 7. L. W.	166	135	4.2	144	156	12
Case 8. L. L.	176	132	7.0	144	154	10
Case 9. V. K.	313	136	8.0	149	167	18
Case 10. L. C.	212	125	5.0	135	150.5	16
Case 11. E. S.	204	150	3.4	158.4	158.5	0
Case 12. K. N.	239	138	4.9	148	151	3
Case 13. W. M.	294	138	7.0	150	151	1

* Ca estimated as 5 mEq./L.

Case Reports. CASE 1., R.B., was a 5-year-old boy with acute hemolytic anemia and resulting nephrosis who died after 9 anuric days. In his case it is puzzling that although his blood urea nitrogen (BUN) rose progressively to 850 mg. per 100 ml. on the last day, the retained unknown substance rose progressively to the 7th day of illness and then fell to normal limits prior to death. Terminal therapy consisting of retention enemas of $NaHCO_3$ and ion exchange resins may have in some way led to the excretion of the unknown substance into the bowel.

CASE 2, D.B., was a 5½-year-old girl with acute nephritis who suffered a 5-day episode of anuria during which the 3 determinations shown in Table 2 were made. At no time did this child appear very toxic, although some circulatory embarrassment occurred, and 2 months following diuresis she appeared fully recovered, with a nearly normal urine.

CASE 3, N.R., was an 11-year-old boy admitted 12 hours after the onset of illness with anuria, diarrhea, coma, and convulsions, who died on the 7th day without regaining consciousness. The question of tetanus was raised on admission, so large amounts of tetanus antitoxin were given and a small leg wound was debrided. At necropsy, there was lower nephron nephrosis, possibly myoglobinuric, uremic colitis, and edema of the brain. The determination shown in Table 1 was made on the 3rd day of illness.

CASE 4, R.J., was a 12-year-old boy with terminal nephritis without oliguria; 10 determinations were made during his last 2 months of life. The difference of 13 mEq. per L. occurred during a 2-week period in which he had an unusual amount of vomiting, but the vomiting continued after the difference disappeared, so a specific clinical correlation is impossible.

CASE 5, P.De W., was a 7-year-old girl with pyelonephritis and obstructive uropathy secondary to bladder neck obstruction, who died in uremia and cardiac failure. The determination noted was made 4 months prior to death

during a severe uremic episode in which constant indwelling catheter with irrigation prevented urine output estimate, but definite oliguria was not present.

CASE 6, L.W., was a 4-year-old girl with temporary 8-day renal shutdown following an injection of bismuth. Diuresis began the day following admission, and the values shown were obtained during this period. Vomiting without nausea was present, but no other signs of toxicity.

CASE 7, L.W., was an 82-year-old man who died in uremia and renal failure secondary to prostatic hypertrophy and obstructive uropathy. He was in desperate clinical condition with uremia, anemia, diarrhea and decubiti when the determination noted was made, but was not anuric.

CASE 8, L.L., was a 43-year-old man who died after 10 years of admissions for nephrocalcinosis secondary to parathyroid carcinoma. The determination noted was made in his last month of life, at which time he was severely ill, anemic and uremic, but not oliguric.

CASE 9, V.K., was a 43-year-old man with Buerger's disease who died of thrombosis of the aorta at the level of the renal arteries. Acute renal failure and oliguria occurred in spite of removal of thrombus and sympathectomy, and he died on the 6th postoperative oliguric day. At necropsy the left renal artery was occluded and the kidney infarcted; the right renal artery was partially occluded, and there was a right renal infarct.

CASE 10, L.C., was a 36-year-old woman who died of chronic glomerular nephritis with malignant hypertension and uremia. The determination in Table 1 was made during her terminal illness.

CASES 11, 12, and 13 were cases of fatal chronic glomerulonephritis. The blood urea nitrogen was very high, but there was essentially no difference between total base and the sum of Na, K, and Ca. See Table 1.

The only substances detected in appreciable amounts by chromatographic study of plasma extracts of these uremic patients were creatinine or guanidine, or both. The R_f of creatinine is only slightly greater than that of guanidine, hence no definite differentiation can be made between the two. This at least indicates that the method for the preparation of plasma extract was effective for the extraction of guanidine and creatinine.

The chromatographic studies were initiated only after the striking difference between the total base and inorganic cations had been observed, so that the advantage of detecting relatively large amounts of the unknown substance(s) as compared to Na in the plasma extract has been lost. The relatively large sodium spot on the chromatogram could easily obscure a substance of similar R_f value but present in relatively low concentrations, as the unknown substance(s) might well be, in the plasma extract.

The yield of cations by known nitrogenous substances upon electrodialysis was assayed by determining the total base values of pure solutions containing 100 millimoles per liter of each of the following substances in the same manner as the plasma total base was determined. The total base values are: guanidine, 162; arginine hydrochloride, 100; lysine hydrochloride, 95; creatinine, 59; alanine, asparagine, citrulline, and histidine hydrochloride, all nil.

Discussion. The only common denominator in these patients is severe renal disease either acute or the last stages of various chronic renal disorders. The unknown substance(s) is present only in very sick patients, and generally when urinary output is inadequate, due to a variety of causes, that is, hemoglobin or myoglobin casts, renal infarcts, nephrocalcinosis, and severe nephritis. It has not been detected in other situations with tissue breakdown (fever, burns, septicemia, and so forth), or where protein metabolism is defective, as in liver disease; the 60 samples of the control group include patients with these and other conditions. With recovery from renal shutdown, the unknown substance(s) is either again excreted or metabolized. Complete ionic diagrams were obtained on the same plasma samples and our findings were in general agreement

with a previously reported study of renal insufficiency (Sunderman[10]) hence they are excluded from this report. Although the acid residue, "R," might be used to verify whether the elevated total base was present *in vivo* the fact that this component of the plasma anions is often elevated in cases where no difference is observed between total base and the sum of cations prevents one from making use of this calculated value. Case 2 shows that the presence of unknown cation(s) does not necessarily indicate an irreversible clinical course.

The attempts to identify the unknown substance(s) by paper chromatography described above have thus far been unsuccessful. By virtue of the fact that in the procedure for the determination of total base by electrodialysis the unknown substance(s) migrates in an electric field and forms an amalgam with the mercury cathode, one can limit the possible substances to amines or imines and substances that yield these nitrogenous bases through electrolytic reduction. Thus, the chromatographic technique was justifiably aimed at the isolation of amines. Olsen and Bassett concluded from their study on a large number of uremic patients that although guanidine was elevated in uremia the range was wide and no correlation could be made with the clinical picture (Olson and Bassett[5]). They reported guanidine levels in severe uremics of less than 3 mg. per 100 ml. blood. Even if one assumes a level of 10 mg. per 100 ml. of blood, thus 2.7 mEq. per L., all cases in our study show a considerably greater amount of unknown substance(s) to be accounted for, and it is hard to imagine a guanidine accumulation of such magnitude. What is said for guanidine also holds true for creatinine because the highest blood creatinine level found in a study of this substance in nephritis and urinary obstruction was 23 mg. per 100 ml. (Cantarow and Davis[2]). Even assuming a level of 40 mg. per 100 ml. this yields only a little over 2 mEq. per L. of total base. Therefore, contributions to total base value from retained creatinine and guanidine could not possibly exceed 5 mEq. per L. As we have shown that high concentrations of urea added to plasma do not contribute to the total base value, one cannot account for the difference between the determined total base values and the sums of Na, K, and Ca on the elevation of known nitrogenous substances that are present in uremia in significant amounts. Ammonia, due to its high toxicity in minute amounts (1 to 1.5 micromoles per liter produce central nervous system symptoms) cannot be a significant factor in this phenomenon. Although amino acids such as arginine and lysine yield nitrogenous base on electrodialysis the patients studied did not show such liver damage as to allow a significant elevation of the concentration of these amino acids in blood (Kirk[3]). The presence of unidentified substance(s) retained in uremia has been suggested as a working hypothesis (Patch and Rabinowitch[6]); however, positive identification or at least the isolation of a specific entity is still needed. Inasmuch as our chromatographic studies of the plasma extract have thus far failed to show a distinct chemical entity we must consider the following possibilities: 1) The unknown substance retained by the unusual uremic patients would yield cations only on electrodialysis through electro-reduction. 2) The unknown substance was not extracted by the method used because of firm bonding with the precipitated plasma proteins but is nevertheless a true nitrogenous base. 3) The unknown substance was extracted in the form of free nitrogenous base but has a R_f

similar to Na, K, guanidine, or creatinine and hence escaped our detection.

Since no direct correlation has been established between a known chemical entity in the uremic blood and the clinical symptoms (Olson and Bassett[5]) our study points to the possibility that some of the symptoms of uremia might yet be explainable on the basis of accumulation of substances normally not present in detectable amounts.

Summary. Blood plasma from 10 patients with severe renal disease showed a significant difference up to 40 milliequivalents per liter between total base by electrodialysis and the sum of sodium, potassium and calcium. The difference is not found in all uremic patients and cannot be accounted for by the accumulation of known nitrogenous bases. An unsuccessful attempt at the isolation and identification of the unknown substance by paper chromatography was made.

REFERENCES

1. **Archibald, R. J.:** J. Biol. Chem., **157**, 507, 1945.
2. **Cantarow, A.,** and **Davis, R. C.:** J. Lab. & Clin. Med., **18**, 502, 1932.
3. **Kirk, E.:** J. Clin. Invest., **12**, 1091, 1953.
4. **Murayama, M.:** J. Lab. & Clin. Med., **33**, 906, 1948.
5. **Olson, N. S.,** and **Bassett, J. W.:** Am. J. Med., **10**, 52, 1951.
6. **Patch, F. S.,** and **Rabinowitch, I. M.:** J. Am. Med. Assn., **90**, 1092, 1928.
7. **Reardon, H. S., Graham, B. D., Wilson, J. L., Baumann, M. L., Tsao, M. U.,** and **Murayama, M.:** Pediatrics, **6**, 753, 1950.
8. **Schwyzer, R.:** Acta chem. Scandinav., **6**, 219, 1952.
9. **Sobel, A. E., Rockenmacher, M.,** and **Kramer, B.:** J. Biol. Chem., **152**, 255, 1944.
10. **Sunderman, F. W.:** New York State Med. J., **53**, 57, 1953.
11. **Tsao, M. U.:** Scand. J. Clin. & Lab. Invest., **2**, 102, 1950.

SUMMARIO IN INTERLINGUA

Differentias Inter Le Valores Del Base Total E Del Summa Del Cationes in Le Plasma De Patientes Con Sever Morbo Renal

Le plasmas del sanguine de 10 patientes con sever morbo renal monstrava significative differentias (amontante a usque a 40 milliequivalentes per litro) inter le valores del base total (determinate per electrodialyse) e le summas de natrium, kalium, e calcium. Tal differentias non occurre in omne patientes uremic e non pote esser explicate como resultante del accumulation del cognoscite bases nitrogenic. Le tentativa de isolar e identificar le incognoscite substantia per chromatographia a papiro non succedeva.

PROGRESS
OF
MEDICAL SCIENCE

PEDIATRICS

UNDER THE CHARGE OF

IRVING J. WOLMAN, M.D.
DIRECTOR OF LABORATORIES, CHILDREN'S HOSPITAL OF PHILADELPHIA
ASSOCIATE PROFESSOR OF PEDIATRICS, UNIVERSITY OF PENNSYLVANIA

AND

ALFRED M. BONGIOVANNI, M.D.
DIRECTOR, ENDOCRINE DIVISION, CHILDREN'S HOSPITAL OF PHILADELPHIA
ASSOCIATE PROFESSOR OF PEDIATRICS, UNIVERSITY OF PENNSYLVANIA
PHILADELPHIA, PENNSYLVANIA

STUDIES ON ELLIPTOCYTOSIS
I. HEREDITARY ELLIPTOCYTOSIS IN THE PEDIATRIC AGE PERIOD: A REVIEW OF RECENT LITERATURE

BY IRVING J. WOLMAN, M.D.

AND

AYŞE ÖZGE, M.D.

(From the Hematology Laboratory, The Children's Hospital of Philadelphia, and the Department of Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania)

HEREDITARY elliptocytosis (ovalocytosis, familial elliptocytosis, constitutional elliptocytosis, genotypic elliptocytosis) has been the subject of an abundant body of medical literature since first described by Dresbach[9] in 1904. Good general reviews in more recent years have been published by Lambrecht[24] (1938), Wyandt, Bancroft and Winship[42] (1941), Guasch and Raichs[16] (1948), Heilmeyer and Begemann[18] (1951), Bernard, Dugas and Cotlenko[2] (1953), and Dacie[6] (1954). The red cells exhibit the characteristic deformity from infancy through senescence, but the condition is usually subclinical and goes unrecognized except when specifically searched for. Only few of the published studies have appeared in pediatric journals, and the standard textbooks of pediatrics give it but brief mention. For this reason, and because of the current wave of interest in hereditary dystrophies of red cells and hemoglobin, it seemed worthwhile to prepare a review of recent reports which gives special attention to the manifestations in the pediatric age period.

Prevalence. Elliptocytosis was encountered 4 times by McCarty[29] in the routine study of 10,000 Americans;

2 times by Wyandt *et al.*[42] in 7,000 Americans; and 4 times by Bernard, Dugas and Cotlenko[2] in 8,000 examinations of a French population. Thus, as a generalization, elliptocytosis may be said to have a prevalence of approximately 0.03 to 0.05% in white populations. Its frequency in other racial groups has not been estimated, to our knowledge, but individual cases have been reported from all corners of the world. In its ubiquitous distribution this disorder resembles hereditary spherocytosis more than Mediterranean anemia or the variant anomalies of hemoglobin. Since elliptocytosis can be recognized by simple examination of a blood smear, population surveys are not difficult to perform.

Genetics. Scores of human pedigrees have been published which trace the existence of elliptocytosis through successive generations of individual families. Practically without exception the anomaly has been found to be transmitted as a Mendelian dominant, not sex-linked. When one parent is affected, which is the typical situation, the trait appears in approximately one-half of the offspring.

This pattern of genetic behavior, in which typical blood cell changes can be detected in the heterozygote, is the same as that of hereditary spherocytosis. Like the latter disease also, hereditary elliptocytosis expresses itself with varying degrees of clinical severity in different heterozygotes, though on the whole there is a much lower incidence of anemia and hemolytic phenomena. Penfold and Lipscomb[31], from a survey of 400 reported cases, estimated that approximately 12% of affected subjects have detectable evidences of increased hemolysis.

A very few apparently homozygous individuals with hereditary elliptocytosis have been recorded (see below).

Of interest are the many reports of families with hereditary elliptocytosis in whom this condition has been associated with some other genetically transmitted anomaly such as hemorrhagic telangiectasia[31], divergent strabismus[15], bone deformities[24], or microspherocytosis[20].

LINKAGE. With the sole exception of the genes of the Rh system, the elliptocytic trait has not been found to exhibit linkage or association with any of the other known attributes of the human erythrocyte. Morton[30] recently surveyed 14 elliptocytic pedigrees collected from the literature in which blood grouping data were available for all or nearly all members of affected families. In the group as a whole the statistical evidence for linkage between elliptocytosis and some one Rh type was "enormously significant," though there was no association between the elliptocytosis and any single Rh blood type. Among the 7 pedigrees with an adequate number of progeny for proper analysis, 3 showed no evidence of linkage whereas 4 others exhibited close though not universal linkage. A good example of this linkage phenomenon is the family surveyed by Marshall, Bird, Bailey and Beckner[28]. In 26 members studied, 15 to 85% of all circulating erythrocytes were elliptic in 12 members. The 11 of these 12 who were surveyed for blood group status all exhibited the Rh pattern of CDe (R_1), whereas only 2 of the unaffected members with an affected parent had this Rh pattern.

Erythrocyte Morphology. WET PREPARATIONS. When erythrocytes of affected individuals are suspended in saline or nonagglutinating human serum and inspected between a glass slide and a coverslip or in a hanging-drop preparation, the external outlines of an appreciable proportion are seen to be elliptic or oval instead of circular. All of the eccentric cells may ex-

hibit the deformity to approximately the same degree, or the distortions in shape may be more intense in some cells than in others. Sometimes the long dimension of the cell is 3 to 4 times the width, giving it a rod-like or bacilliform shape. The percentages of deformed cells and the intensity of the distortions may not always seem the same in successive examinations.

In the absence of anemia, the distribution of hemoglobin within elliptic cells is not abnormal. Most appear normochromic, but occasional ones may exhibit either hypochromia or hyperchromia.

When viewed edgewise, elliptic cells from heterozygotes show the normal biconcave silhouette, though some may be deeply concave in one direction only and have the shape of a coracle or elongated basket[3].

DRIED FILMS. With heterozygotes in smears of dried blood films the anomaly is said to be less marked and the proportion of obviously distorted cells usually lower than in moist suspensions. Severely deformed cells may not readily form the small rouleaux-like agglomerations so often seen in films of normal disc-shaped red cells. Basophilia and crenation are not characteristic accompaniments. Anisocytosis and poikilocytosis may be seen in the more extreme cases. Central pallor may be prominent when iron deficiency is associated.

CLASSIFICATION OF ELLIPTIC CELLS. Many systems of classification based on the ratio of width to length have been proposed, for the purpose of describing the degree of elliptocytosis in quantitative terms. Widely used is the approach of Günther[17], in which one measures accurately a large number of red cells in microns and calculates the eccentricity from the formula $E = \sqrt{1-\left(\frac{\text{short diameter}}{\text{long diameter}}\right)^2}$. The cells are then subclassified according to the following scheme:

Group I: E from 0.0 to 0.47 (circular)
Group II: E from 0.47 to 0.62 (rounded)
Group III: E from 0.62 to 0.74 (elliptic)
Group IV: E from 0.74 to 1.0 (elongated elliptic)

The percentage of cells in each stage of eccentricity is utilized as an indicator of the severity of the deformity in the individuals examined.

Günther's quantitative approach is time-consuming and gives results of higher precision than are needed in clinical situations. For practical purposes it is enough to count and grade qualitatively all the red cells in one or more microscopic fields.

The discordances which may be encountered in the progressive stages of round (circular), oval (elongated), elliptic, and rod-shaped (elongated elliptic) cell form distribution are illustrated by the findings of Motulsky, Singer, Crosby and Smith[30a] in 3 adult subjects:

Subject	*Clinical Symptoms*	*Round Cells*	*Oval*	*Elliptic*	*Rod-Shaped*
1	0	55%	32%	8%	5%
2	++	10%	6%	76%	8%
3	0	3%	34%	50%	13%

The above 3 subjects were from different families, but even within the same family there may be marked differences in the percentages of misshapen forms among the various members.

Ovalocytosis from Other Causes. It is a commonplace observation in hematology that oval cell forms are often found within the circulation as one manifestation of poikilocytosis in anemia due to almost any cause. This symptomatic phenomenon has been noted specifically[30a] in pernicious anemia, severe iron-deficiency anemia,

Cooley's anemia, hereditary spherocytosis, some forms of nonspherocytic hemolytic anemia[36], and severe instances of infection, leukemia and cancer. In these disorders the majority of such cells are only moderately elongated, corresponding to Group II of Günther's classification. Frequently they appear asymmetrically distorted, with one pole broader than the other, and occur nearly always in the company of other more bizarre-shaped cell forms. Long rod-shaped forms are very uncommon as a manifestation of poikilocytosis, but may be abundant in hereditary elliptocytosis. Attention may be directed to the frequent occurrence of oval cells and "microelliptocytes" in hereditary blood disorders related to Mediterranean anemia[7a,27].

Even in healthy subjects who have no known anemia of any sort it is not unusual to see oval-shaped forms within their erythrocyte population. The studies of Wyandt *et al.*[42] of 450 students and nurses showed the following frequencies of this finding:

Percentage of Students and Nurses	*Percentage of Elliptic Red Cells*
58	1 or less
37	2 to 5
4	5 to 10
1	10 to 15

Criteria for Diagnosis of Hereditary Elliptocytosis. PERCENTAGE OF ABNORMAL FORMS. Unanimity is lacking among the experts with respect to the threshold number of eccentric cells which is necessary for one to diagnose the existence of elliptocytosis. Minimal values which have been proposed are Lambrecht[24], 10%; Florman and Wintrobe[11], 40%; Wyandt, Bancroft and Winship[42], 25%; Heilmeyer and Begemann[18], 10 to 20%.

Certain considerations influence this problem of setting a dividing line between normals and carriers of hereditary elliptocytosis[2]:

1. The exhibition of the characteristic may be influenced by technical factors, sometimes obscure. For example, to quote Wyandt[42], "On one day, one of four preparations had twice as many sausage forms as the other three mounts made at the same time." The pressure exerted in the making of dried smears can either obscure or accentuate the deformity.

2. The proportion of elliptocytes in the circulation often varies in successive examinations. This may be the result of technical factors or of biologic influences of unknown nature.

3. The characteristic deformity in shape may be accentuated by coincident anemia. Bernard[2] advises that when authors give a percentage range for differentiation between normal erythrocyte populations and those of hereditary elliptocytosis, the lower figure should apply for healthy subjects and the upper figure for patients with anemia.

4. Within affected families wide divergences are found with respect to percentages of abnormal cells and the presence or absence of symptoms. In some members the abnormal forms may be so few and the deformity so mild that the diagnosis would be overlooked, were it not for the family history and perhaps also the occurrence of a few rod-shaped forms.

Importance of Family Survey. With all suspected and questionable cases it is important to study as many members of the family as possible for the existence of the trait. With infants and children the examiner should study both parents, all siblings, and as many of the grandparents and collateral members of both sides of the family as will cooperate.

When the family survey is negative but an individual nevertheless seems to

have elliptocytosis of the hereditary type, one must assume either that a mutation has occurred or that the characteristic was inherited from a parent in whom the penetrance of the gene has been incomplete. To exclude the condition of acquired ovalocytosis in such an individual it is important that the elliptic erythrocytes persist in significant numbers over a period of years, without any other known cause of the oval deformity being demonstrable.

Hematologic Properties of Elliptic Cells. The peculiar shape of the red cells in hereditary elliptocytosis has inspired many experiments designed to elucidate the condition by finding other deviations from the normal. The resemblance of these cells to the oval-shaped *biconvex* erythrocytes of the Camelidae (camel, dromedary, guanaco, llama, vicuna) is apparently restricted only to their contours[2].

When the disorder is entirely latent the red cells tend to have a normal mean volume and a normal mean content of hemoglobin with little individual deviation from these means. When there is a superimposed iron deficiency the values are lower.

Occasionally, as in the family reported by Stephens and Tatelbaum[37] (which included 2 affected children), there may be an increase in the count of cells per cubic millimeter with a diminution in mean cellular volume and mean cellular hemoglobin ("microcytosis"); in this family, two-dimensional measurements indicated that the abnormally shaped cell forms had approximately the same size as the round cell forms.

When a hemolytic process is present both the elliptic and the round erythrocytes are likely to exhibit some degree of anisocytosis, poikilocytosis and central achromia.

Target cell forms have been extremely uncommon in most reported instances, regardless of whether the disorder was latent or symptomatic. Unlike sickle cells, elliptic cells do not alter their shape in the presence of a high plasma CO_2 content[2], save when there is an unrelated anomaly of the hemoglobin as well.

OSMOTIC FRAGILITY. The osmotic behavior of the cells in graded hypotonic solutions is usually the same as that of normal disc-shaped erythrocytes, though often a minority of the cells may seem unduly fragile or unduly resistant. However, in some of the patients with erythrodestruction the bulk of the cells have seemed unusually fragile. Kirkegaard and Larsen[23] state that many elliptocytes revert to normal shape when placed in hypotonic or acid or alkaline environments, but other authors[35,38] state that this reversion to the disc form takes place in only a few of the cells and only in hypotonic solutions.

RETICULOCYTES. The range of reticulocytes in normal healthy children is 0.5 to 1.0%[41]. In elliptocytic individuals with hemolytic phenomena the reticulocyte count is usually well elevated, in the range of 2 to 10%. Most asymptomatic childhood cases exhibit a slight increase: the usual reading seems to be between 0.5 and 2.0%, and usually about 1.0%. According to most authorities these younger cell forms are all disc-shaped, but according to Lendval[25] and in our experience a minority sometimes may be oval or even elliptic.

BONE MARROW. In asymptomatic individuals the bone marrow exhibits no increase in the rubricytes or other cell series. When hemodestruction is active, the rubricytic components are increased in quantity but appear qualitatively normal. In the literature the statement is made almost universally that megaloblasts, normoblasts and other nucleated red cell forms have no shape distor-

tion. A contrary opinion is expressed by Franzini[12], whose measurements showed that even in aspirates from normal subjects many of these immature cells may be slightly oval, but that in hereditary elliptocytosis the characteristic asymmetric deformity is much more prominent.

LIFE SPAN. From the published studies[22,23,30a,39], it would appear that elliptic cells from asymptomatic subjects have an undiminished survival time when transfused into other individuals, whereas elliptic cells from patients with an active hemolytic process have a reduced survival time. For example, in the 9-month-old infant with active hemolysis described by Josephs[22] the 50% survival time was only 16 days. In the adult with hemolytic symptoms studied by Motulsky and associates[30a] the total survival time seemed to be only 45 days instead of the normal 100 to 120 days, whereas in two other asymptomatic adults the survival time was over 100 days.

FRAGMENTATION. When mature erythrocytes from diverse varieties of anemia are kept warm in wet coverslip preparations on a slide, occasional cells may show bud-like protrusions or central constrictions, with the eventual formation of two or more disconnected rounded formations of hemoglobin-containing cytoplasm. This phenomenon is a feature also of Cooley's anemia, sometimes rather prominently[5].

In Lipton's[26] apparently homozygous infant, some of the elliptic red cells when suspended in physiologic saline solution exhibited "the process of splitting as if reproducing by fission." We have observed a similar phenomenon in a similar apparently homozygous infant as yet unreported.

OTHER HEMATOLOGIC PHENOMENA. When erythrodestruction and erythroproduction are accelerated the total serum bilirubin level may be elevated moderately, with the 1-minute or "direct" value being unaffected. Readings between 1.0 and 2.5 mg. per 100 ml. often have been noted in such cases[2].

Elliptic cells retain their distinctive shape in the blood stream of normal recipients, showing that the lesion is intrinsic in the stoma rather than a response to some factor in serum. Normal red cells do not become elliptic when suspended in the plasma of a patient.

Hemoglobin electrophoresis reveals a normal AA pattern, although a few instances have been recorded in which an abnormal hemoglobin was present as a coincidental anomaly[1] (see below). The fetal hemoglobin was 0.55% of all hemoglobin in a 6-year-old girl with asymptomatic elliptocytosis studied by Bernard *et al.*[2]. Serum iron levels in asymptomatic cases are typically within normal limits. Stool urobilin content was at the upper limit of normal in 3 children with hemolytic elliptocytosis studied by Josephs[22]. Coombs tests and related tests for circulating antibodies are regularly negative[6]. Elliptic red cells studied *in vitro* may show diminished reduction of autohemolysis in the presence of added glucose[36].

Unpublished studies recently carried out by this Laboratory in conjunction with Dr. Arthur Parpart and Earl Van Norman of Princeton University indicate that nonhemoglobin-containing elliptic red cells ("ghosts") and sections of whole red cells of heterozygous individuals when studied by electron microscopy show fewer deviations from the normal than do the cells of a homozygote. The latter exhibit a variety of cytoplasmic variations from both the heterozygote and the normal.

Clinical Expressions. Nearly all instances of hereditary elliptocytosis which are on record have been found

by family surveys to be heterozygotes. Only a very few apparent homozygous individuals have been discovered: these are described separately in a later section. Accordingly, except where otherwise specified, the comments in this Review are directed toward the manifestations of this disease in heterozygotes.

On the basis of the absence or severity of erythroproduction and erythrodestruction, the disturbance in affected individuals has been subclassified as: (1) *latent* or *inactive;* (2) *compensated;* or (3) *symptomatic.* Affected families usually contain individuals who fit into each of these three groups.

LATENT DISTURBANCE. An estimated 88% of elliptocytic subjects are free from detectable physiologic evidences of anemia or of increased erythroproduction or erythrodestruction. In these individuals the existence of the anomaly is recognizable only through morphologic study of the erythrocytes.

COMPENSATED DISTURBANCE. This term is applied when individuals exhibit clinical and laboratory signs of a moderate increase in erythrodestruction, not sufficient to overtax the compensatory mechanisms which regulate erythroproduction. There is rubricytic hyperplasia in the bone marrow, slight elevation of the total but not of the 1-minute serum bilirubin level, a mild reticulocytosis, and normal or augmented excretion of urobilinogen and related pigments in stools and urine. The peripheral blood may show a mild or minimal diminution in level of circulating hemoglobin and erythrocytes. When the iron intake is inadequate the anemia may be hypochromic rather than normochromic. The spleen tip may be palpable. Gallstones sometimes develop in later life.

SYMPTOMATIC DISTURBANCE. This term is used when erythrodestruction outstrips hyperactive erythroproduction. Anemia, hyperbilirubinemia, reticulocytosis, jaundice, splenomegaly and other signs of excessive hemolysis become apparent, though often intermittently. Acute "crises" of sudden hemolysis have been described.

Most of the reported patients with overt signs of such pronounced hemolytic activity have been adults. The reports often include sketchy data on the other members of the families, including children, but only a limited number of infants and young children have been described in detail. A few recently reported examples follow.

Dacie, Mollison, Richardson, Selwyn and Shapiro[7] described a 10-day-old elliptocytic infant who was ill with a rapidly progressive hemolytic anemia. Transfusions had to be given at approximately monthly intervals until splenectomy was done at age 6 months. Thereafter the anemia persisted, but hemodestruction was much less marked and further transfusions were not needed. Post-splenectomy blood examinations showed marked anisocytosis and many microspherocytes and irregular cell fragments. The father's red cells seemed normal, but nearly all those of the mother and brother (neither was anemic) were oval or elliptic.

Cathie and Goldsmith[3] (1953) recorded a 7-year-old British girl who had sporadic episodes of abdominal pain, vomiting and jaundice in association with dark feces since 2½ years of age. When examined, she had a normochromic anemia, a total serum bilirubin of 2 mg. per 100 ml., a reticulocytosis of 2 to 5%, and a high percentage of elliptocytes in wet preparations. Her spleen, which extended ½ inch below the costal margin, was removed surgically. On pathologic examination this exhibited only congestion and slight excess of hemosiderin (similar minimal changes have been

the rule in pathologic reports from other cases). Following operation the vomiting and jaundice disappeared but the elliptocytosis, normochromic anemia and mild hyperbilirubinemia persisted. A few other similar instances have been reported in both children and adults, in whom splenectomy was followed by amelioration of symptoms without much change in the hematologic picture.

Josephs and Avery[22] (1955) described 3 male Negro infants with hereditary elliptocytosis and signs of increased hemolysis. One of these was discovered at the age of 2 months with a hemoglobin of 5.5 gm. per 100 ml., red cell count 2,860,000, reticulocytosis of 4.9%, normoblastic increase in the bone marrow, and pronounced elliptocytosis with some poikilocytosis in the blood smear. Another elliptocytic child presented at the age of 5 months with the general picture of iron-deficiency anemia which over the next 2 years was resistant to iron therapy. A third case, first found to be anemic at 9 months, was interpreted as having iron deficiency anemia responsive to iron therapy in association with prominent elliptocytosis. The red cells of this last infant, when studied with radioactive chromium (Cr_{51}), had only a 50% survival after 16 days.

Influence of Age. Hunter[21] described an asymptomatic infant born to an elliptocytic mother who was studied from birth through 12 days of life and again at 3 months of age. In fresh preparations made of cord blood and in the neonatal period "a fair percentage of erythrocytes were oval and a few had a rodlike form. The exact number of each was not determined. The dried film contained a small number of oval cells but almost no greatly elongated or typical ellipses." In a later fresh specimen, obtained at 3 months of age, there were 44.4% oval cells and 1.6% elongated or irregular poikilocytes. "Many of the erythrocytes were now oval and in stained smears a fair proportion were rodlike with quite bluntly rounded ends." There was "a very obvious increase" in abnormal forms as compared with the first 2 weeks of life.

Helz and Menten[19] followed an elliptocytic infant who had an affected father. The percentage of deformed cells in the infant remained at 25% from 3 months to 4 months, and then rose abruptly to the 65% level where it remained until the infant was last seen at 10 months.

These observations are cited widely as the basis for the generalization that elliptocytosis may be mild or moderate at the time of birth and becomes more prominent in the first few months thereafter. A parallel is often drawn with sickle cell anemia, in which a similar lag in hematologic expressivity may be noted in the postneonatal period. The high neonatal level of fetal hemoglobin (which does not sickle) is believed to be responsible, at least in part, for the inhibition of sickling phenomenon in infants with the sickle cell trait or disease. Since hereditary elliptocytosis appears to be a stromal anomaly, without any evident defect in hemoglobin, the content of fetal hemoglobin within the elliptic red cells cannot be expected to suppress this shape peculiarity.

Inasmuch as technical factors or spontaneous variability may have been operative in Hunter's and Helz and Menten's single patients, it would seem desirable to withhold judgment on any hypotheses concerning neonatal inhibition of elliptocyte formation until after a number of such infants have been studied hematologically and such observations validated.

The Homozygous Condition. Ideally, to establish an affected individual as a homozygote, it must be proven that

both parents possess the anomaly, that all offspring of marriage between the individual and a normal spouse give heterozygously affected children, and (from analogy with other disturbances) that the clinical and hematologic expressions of the disorder are more developed than in the usual heterozygote. All these conditions are not always fully establishable, though ancillary data may be suggestive.

Examples of such presumed homozygotes are very few. Undritz[40] has described an affected grandfather with hemolytic symptoms whose parents were first cousins. In this man all the circulating erythrocytes were elliptic and severely deformed. All 3 of his children and 2 of 5 grandchildren had asymptomatic elliptocytosis, with much less pronounced alterations in their red cells.

Wyandt *et al.*[42] mention a family in which both parents exhibited the anomaly. Two of their children had normal erythrocytes and 2 died without the blood being studied. The fifth child showed 95% elliptic cells, spherocytes, splenomegaly, increased osmotic fragility of the red cells, 2.3% reticulocytes, skin icterus, and "crises" of acute hemodestruction.

In Lipton's[26] infant, jaundice and anemia became evident during the first months of life. The parents were third cousins of Italian descent. Both parents, the mother's mother, the father's father, and a few others in the immediate family had elliptocytosis, with 60 to 75% of the cells being clearly altered; none of these exhibited anemia or evidences of hemolysis. The infant, however, had overt recurrent normochromic anemia and signs of excessive red cell destruction. From 50 to 70% of the circulating red cells were elliptic and the remainder exhibited anisocytosis and poikilocytosis; achromia and round target forms were also noted. There was marked erythroid hyperplasia of the bone marrow, hyperbilirubinemia, increased serum iron, and increased output of fecal urobilinogen. Immunologic studies were negative. The fetal hemoglobin content of the red cells was not elevated, and their osmotic fragility not increased. There was no sickling, and electrophoretic studies showed no abnormal hemoglobin. Normal donor cells transfused into the infant had a normal survival time. Splenectomy resulted in marked but not complete alleviation of the hemolytic phenomena; the red cell morphology was little altered.

Another hematologic feature in Lipton's infant was microcytosis. Approximately 20% of all red cells had lengths of 6.9 microns or less, and of these approximately half were elliptic. In none of the other members of this family did the microcytes exceed 5% of all circulating red cells and most of the smaller cells had lengths not much less than 6.9 microns. In all these other members the elliptocytes were homogenously uniform in contour, without the extreme distortions displayed by the infant.

Hybrid Combinations. There is no *a priori* reason for assuming that elliptocytosis may not occur occasionally by chance in association with some other hereditary blood dyscrasia. A few instances have already been identified, as summarized below. In these the results of the association seem to be largely additive rather than synergistic. Many more established instances of elliptocytosis occurring in association with other genetic anomalies of the red cells and hemoglobin will have to be analyzed before the complete pattern of the hematology of such hybrid disturbances can be elucidated.

Numerous authors have suggested that the exhibition of signs of undue

hemolysis, as found in a minority of apparently heterozygotic elliptocytics, may result from either of two genetic mechanisms. There may be a pronounced degree of expressivity of the trait in heterozygotes, as exemplified by the hemolytic symptoms with microspherocytosis found in three different branches of the family described by Holst-Larsen[20]. As Dacie[6] points out, it would be most improbable that the gene for elliptocytosis would occur in the homozygous state in each of the three branches. The other possibility is that the gene for elliptocytosis may be combined with that of some other genetic anomaly (or anomalies) as yet unrecognized. Thus Lipton[26], impressed with the great numbers of microspherocytes in his apparently homozygous patient, has proposed that this phenomenon results from "a genetic determinant other than the gene for ellipticity."

HEMOGLOBIN C TRAIT. Avery[1] reported 2 Negro children, brother and sister, who exhibited simultaneously both hereditary elliptocytosis and the hemoglobin C trait. The girl, who also had mild lead poisoning, was studied from the ages of 20 to 27 months. Her blood hemoglobin level rose from 6.6 gm. to approximately 10 gm. in 3 months with oral iron therapy and then remained at that level. There was no persistent reticulocytosis, and the red cell survival time using radioactive Cr_{51} was within the range of normal. The boy had a low hemoglobin level at age 3 years, but 2 months of iron therapy raised this to 10.8 gm. When he was studied more intensely at 7½ years of age he had a hemoglobin level of 11.4 gm. Both of these children exhibited numerous elliptic cells in the blood smears and showed the electrophoretic pattern of hemoglobin C trait as well. Neither had hepatosplenomegaly, icterus, or evidences of accelerated hemolysis. The father's blood contained about 15% elliptic cells; he was not anemic. The mother, who carried the hemoglobin C trait, had no elliptocytes. One other sibling, with marked elliptocytosis and a normal electrophoretic hemoglobin pattern, exhibited a mild well-compensated hemolytic tendency.

SICKLE CELL TRAIT. Faden[10] has reported a 25-year-old Negro male with persistent mild anemia who presented the peripheral blood picture of elliptocytosis along with the sickle cell trait. Sickling of the "holly-leaf" type occurred only in the normal-appearing discoid red blood cells. In the blood smears his red cells exhibited "65 to 84% ovalocytes, 3 to 11% sickled cells, and some normal appearing discoid cells. The red blood cell counts and blood indices were within normal limitation. The red blood cells showed an increased resistance to hypotonic saline solutions." A family survey and hemoglobin electrophoresis studies were not made.

Therapy. Hereditary elliptocytosis thus appears to be a wholly benign condition in all but a small percentage of affected individuals. Transfusions may be given when thought necessary for refractory low hemoglobin levels. An augmented iron intake may be advisable in the early years while body growth is rapid, in order to minimize the opportunity for secondary iron deficiency. When erythrodestruction is very active, or when other signs of hypersplenism are evident in the form of granulocytopenia or thrombocytopenia, splenectomy may be considered; the operation may be followed by a partial alleviation of the hemolytic symptoms. No reports have been found on the possible nonspecific effect of adrenocortical hormones in retarding the accelerated hemodestructive process. A long life span is exhibited by nearly all cases.

REFERENCES

(1) **Avery, M. E.:** Bull. Johns Hopkins Hosp., **98**, 184, 1956.
(2) **Bernard, J., Dugas, M.,** and **Cotlenko, M.:** Ann. méd., **54**, 652, 1953.
(3) **Cathie, I. A. B.,** and **Goldsmith, H. T.:** Great Ormond St. J., p. 44, June, 1953. (4) **Chalmers, J. N. M.,** and **Lawler, D. S.:** Ann. Eugenics, **17**, 267, 1953. (5) **Cooley, T. B.:** Am. J. Med. Sci., **209**, 561, 1945.
(6) **Dacie, J. V.:** The Haemolytic Anaemias. London: J. & A. Churchill, Ltd., Chapt. 3, 1954. (7) **Dacie, J. V., Mollison, P. L., Richardson, N., Selwyn, J. G.,** and **Shapiro, L.:** Quart. J. Med., **22**, 79, 1953. (7a) **Dameshek, W.:** Am. J. Med. Sci., **205**, 643, 1943. (8) **Diggs, L. W.,** and **Bibb, J.:** J. Am. Med. Assn., **112**, 695, 1939. (9) **Dresbach, M.:** Science, **19**, 469, 1904.
(10) **Faden, R. S.:** Blood, **4**, 505, 1949. (11) **Florman, A. L.,** and **Wintrobe, M. M.:** Bull. Johns Hopkins Hosp., **63**, 209, 1938. (12) **Franzini, C.:** Le Sang, **21**, 141, 1950.
(13) **Goodall, H. B., Hendry, D. W. W., Lawler, S. D.,** and **Stephen, S. A.:** Ann. Eugenics, **17**, 272, 1953. (14) Iidem: Ann. Eugenics. **18**, 325, 1954. (15) **Guasch, J.:** Acta haemat., **1**, 178, 1948. (16) **Guasch, J.,** and **Raichs, J.:** Symposium de Hematologia y Hemoterapia. Barcelona, 1948. (17) **Günther, H.:** Deutsches Arch. f. klin. Med., **162**, 215, 1928.
(18) **Heilmeyer, L.,** and **Begemann, H.:** Blut und Blutkrankheiten, 4th ed. of Handb. d. Inn. Med. Berlin: Springer-Verlag, 1951, Vol. 2. p. 330. (19) **Helz, M. K.,** and **Menten, M. L.:** J. Lab. & Clin. Med., **29**, 185, 1944. (20) **Holst-Larsen, T.:** Nord. med., **34**, 925, 1947. (21) **Hunter, W. C.:** Ann. Int. Med., **6**, 775, 1932.
(22) **Josephs, H. W.,** and **Avery, M. E.:** Pediatrics, **16**, 741, 1955.
(23) **Kirkegaard, A.,** and **Larsen, K.:** Acta med. Scandinav., **110**, 510, 1942.
(24) **Lambrecht, K.:** Ergebn. inn. Med. u. Kinderh., **55**, 295, 1938. (25) **Lendval, J.:** Lancet, **1**, 582, 1949. (26) **Lipton, E. L.:** Pediatrics, **15**, 67, 1955.
(27) **Marmont, A.,** and **Bianchi, V.:** Acta haemat., **1**, 4, 1948. (28) **Marshall, R. A., Bird, R. M., Bailey, H. K.,** and **Beckner, E.:** J. Clin. Invest., **33**, 790, 1954. (29) **McCarty, S. H.:** J. Lab. & Clin. Med., **19**, 612, 1934. (30) **Morton, N. E.:** Am. J. Human Genet., **8**, 80, 1956. (30a) **Motulsky, A. G., Singer, K., Crosby, W. H.,** and **Smith, V.:** Blood, **9**, 57, 1954.
(31) **Penfold, J. B.,** and **Lipscomb, J. M.:** Quart. J. Med., **12**, 157, 1943. (32) **Ponder, E.:** J. Exper. Biol., **28**, 567, 1951. (33) Idem: Acta haemat., **12**, 282, 1954. (34) Idem: Blood, **6**, 559, 1951.
(35) **Roth, O.,** and **Jung, E.:** Folia haemat., **44**, 549, 1931.
(36) **Selwyn, J. G.,** and **Dacie, J. V.:** Blood, **9**, 414, 1954. (37) **Stephens, D. J.,** and **Tatelbaum, A. J.:** J. Lab. & Clin. Med., **20**, 375, 1934–1935.
(38) **Terry, M. C., Hollingsworth, E. W.,** and **Eugenio, V.:** Arch. Path., **13**, 193, 1932. (39) **Trinick, R. H.:** Lancet, **1**, 963, 1948.
(40) **Undritz, E.:** Le Sang, **25**, 293, 1954.
(41) **Wolman, I. J.:** Laboratory Applications in Pediatric Practice. New York: McGraw-Hill Book Co., Inc., 1957. (42) **Wyandt, H., Bancroft, P. M.,** and **Winship, T. O.:** Arch. Int. Med., **68**, 1043, 1941.

GYNECOLOGY AND OBSTETRICS

UNDER THE CHARGE OF

CARL BACHMAN, M.D.
PROFESSOR OF OBSTETRICS AND GYNECOLOGY, SCHOOL OF MEDICINE
UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PENNSYLVANIA

AND

FRANKLIN L. PAYNE, M.D.
PROFESSOR OF OBSTETRICS AND GYNECOLOGY, SCHOOL OF MEDICINE
UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA, PENNSYLVANIA

EXFOLIATED CYTOLOGY IN GYNECOLOGICAL MALIGNANCY A REVIEW OF THE LITERATURE

BY ROBERT R. RASCOE, M.D.
FELLOW OF THE AMERICAN CANCER SOCIETY

(From the Department of Obstetrics and Gynecology, Hospital of the University of Pennsylvania, Philadelphia, Pennsylvania)

MALIGNANT lesions of the vulva, vagina, uterus, tubes and ovaries exfoliate cells which may be identified in cytological smears. Papanicolaou[86] first reported the recognition of cancer cells in the vaginal smear in 1928. The importance of this discovery and the potentialities of its use in study, diagnosis, and treatment of genital malignancy was not generally recognized until 1941 when Papanicolaou and Traut[91] published their classic paper, "Diagnostic Value of Vaginal Smears in Carcinoma of the Uterus." Since 1941 many investigators have reported on the identification of normal, atypical, and malignant cells exfoliated from the genital tract and the relationship of these cells in the development, diagnosis, and prognosis of gynecological malignancy.

IDENTIFICATION OF NORMAL EXFOLIATED CELLS. Papanicolaou *et al.*[94], Papanicolaou and Traut[92], and others[3, 77, 122] have described the normal cells exfoliated from the female genital tract. Exfoliated cells from the vagina, cervix, endocervix, and endometrium may be identified in the vaginal smear. The vagina and pars vaginalis of the cervix are covered by stratified squamous epithelial cells. The squamous epithelium of the female genital tract is divided into four layers: the germinal layer, the transitional layer or basal layer, the intermediate layer and the superficial layer. The germinal cells are located just above the basement membrane and usually are not exfoliated into the vaginal pool as are the other three types. The basal cells, sometimes called parabasal cells, originate from the transitional layer of the epithelium. The inner basal cells are small and round with a basophilic cytoplasm and a round or oval nucleus. The outer basal cells are also round or oval but somewhat larger than the inner ones. The cytoplasm does not stain as deeply basophilic and the amount of peri-

nuclear cytoplasm is greater. The cells that originate from the intermediate layer of the squamous epithelium are larger than the basal cells and are elongated or elliptical. The nuclei are larger, have a tendency to be elongated and may be pushed towards the periphery of the cell. The cytoplasm is usually basophilic but may be acidophilic. Vacuolization in the cytoplasm is frequent. These cells have been called navicular cells and are prominent in pregnancy. The exfoliated cells that originate from the superficial layer of the squamous epithelium can be divided into three types. These cells are large and flat with an abundance of cytoplasm. The precornified cells have pale basophilic cytoplasm with a vesicular nucleus. The cornified cells have acidophilic cytoplasm and a pyknotic nucleus. The keratinized cells stain orange and the nucleus has disappeared.

The cervical canal is lined by tall columnar epithelial cells with basal nuclei. These cells have been called the "picket fence" type of epithelium. The columnar cells from the endocervix are exfoliated and may be found in the vaginal smears. In some instances they may be well preserved and have the same appearance as in histological sections of the cervix. Cilia may be seen at the end of these well-preserved cells and the cytoplasm stains pink. These cells degenerate rapidly and in many instances only wisps of cytoplasm may remain or, if complete degeneration has taken place, the nucleus appears free of cytoplasm. The nuclei of the endocervical cells are granular with an occasional clump of chromatin and they vary markedly in size.

Endometrial cells may be identified in vaginal smears. They degenerate rapidly and there is only a suggestion of cytoplasm surrounding the nuclei. The cell walls have no definite cellular borders. The nuclei have distinct surrounding membranes, are uniform in size, and appear dark and active with chromatin in small clumps. The cells frequently appear in groups and overlap one another.

Other cells which are seen in the vaginal smear, but which do not originate from the genital tract, are histiocytes, leukocytes, lymphocytes, and red blood cells. The histiocyte may cause some difficulty in identification. The histiocyte has a round, bean shaped, or oval eccentric nucleus which appears to be active because of the small clumps of chromatin. The finely vacuolated or foamy cytoplasm stains green.

IDENTIFICATION OF MALIGNANT CELLS. Papanicolaou and Traut[91] in 1941 described the criteria necessary for the diagnosis of malignancy by vaginal smear. In their monograph in 1943, they described in great detail the characteristics of the exfoliated cells found in uterine cancer[92]. Malignant cells, exfoliated from squamous cell carcinoma of the cervix, have large nuclei with hyperchromasia and clumped chromatin. One or more nucleoli may be prominent and multinucleation is sometimes seen. The nuclei may vary in size and shape and the nuclear membrane may be irregular or bulging. The chromatin frequently collects around the edge of the nucleus to give it a distinct nuclear membrane. The malignant squamous cells may have little or no cytoplasm with ill-defined cell borders or the cytoplasm may be well preserved with a definite cell wall visible.

The exfoliated cells in adenocarcinoma are not as large and as prominent as those seen in squamous cell carcinoma. Grouping of these cells is frequent. The cells may have distinct cellular borders with frequent vacuoles in the cytoplasm. Inclusions of white

cells, plasma cells and cellular debris may be seen in the vacuoles. Other exfoliated adenocarcinoma cells have only a faint and ill-defined cytoplasm with indistinct cell membranes. The nuclei are larger than normal endometrial cells and have a sharp nuclear outline. Variation in size is marked and the nucleoli may vary in number and size. The nuclei are hyperchromatic, sometimes appear black and are eccentric.

The Staff of the Cytological Laboratory of the Vincent Memorial Hospital[122] in their book "The Cytologic Diagnosis of Cancer" have presented a well-organized classification of malignant cells. The malignant squamous cells can be divided into the undifferentiated and the differentiated types. The undifferentiated malignant squamous cells have no visible cytoplasm or only a background of cytoplasm surrounding the malignant nucleus. In the differentiated malignant cell, the cytoplasm is prominent with well-defined cell borders. These cells are divided into three groups depending upon the appearance and shape of the cells. The fiber or snake cell is long and thin, shaped like a smooth muscle cell and contains an elongated, abnormal nucleus. The tadpole cell is large with sharply defined cellular borders and a long tail of cytoplasm. Their nuclei fill the criteria of malignancy. The last type of differentiated malignant cell is called "third type differentiated cell." This is a round cell with a moderate amount of cytoplasm containing a malignant nucleus. The cell wall is well-defined and the nuclear-cytoplasmic ratio is altered from the normal. Other bizarre cells in which the nuclear and cytoplasmic malformations are more pronounced may be found in the differentiated types of squamous cell carcinoma.

Malignant cells of adenocarcinoma may also be divided into undifferentiated and differentiated depending on the prominence of the cytoplasm. Undifferentiated adenocarcinoma cells are found in tight clusters with only a faint background of cytoplasm and the nuclei fill the criteria for malignancy. The differentiated cells appear in loose clusters, have distinct cell walls and the nuclei are eccentric.

Cytological smears in malignant lesions of the cervix or fundus may have certain other generalized characteristics[92,109,122]. Histiocytes, leukocytes, and bacteria are numerous. Blood is seen as red blood cells, fibrin, and blood pigment. Cytoplasm of the benign basal cells may take an acidophilic stain rather than basophilic. Basophilic squamous cells with large benign appearing nuclei are frequently noted under low power in cases of squamous cell carcinoma of the cervix. Liu[65] has shown that in carcinoma of the uterus a greater cornification is noted in the vaginal smear than in patients of the same age groups who do not have genital cancer. The percentage of basal cells is also increased in smears of cervical cancer and with this increase of cornified cells and basal cells there is a resulting decrease of precornified cells[22,66].

METHODS OF SMEAR PREPARATION. The proper technique of obtaining cytological material and the preparation of the smear is most important[77,92,98,122]. For a complete cytological evaluation of the female genital tract, smears should be obtained from the vagina, cervix, endocervix, and endometrium. Each of these slides should be labeled as to their origin because of the aid in identification of the exfoliated cells.

In the earlier reports only the secretions from the vagina were used for cytological study in the diagnosis of gynecological malignancy[77,91,92,93,95,122]. It is important that the patient not douche and the clinician not examine

the patient prior to taking the smear. The patient is placed on the examining table, the labia are spread and an eight-inch glass pipette with a rubber suction bulb is inserted into the vagina. Aspiration from the posterior vaginal fornix is done and the material is spread on a clean glass microscopic slide. The secretion aspirated from the posterior vaginal fornix contains cells which have been exfoliated from the entire genital tract. This continues to be the first step in the collection of material.

Direct cervical smears are of more value in the diagnosis of cervical cancer than those by vaginal aspiration[2,4,29,61,68,85,100,101,109]. Here there is a selectivity of the cells and the cells are better preserved than those exfoliated into the vaginal pool. Several methods are available in obtaining material from the cervix. Ayre[4] devised a wooden spatula which is used to obtain cervical cells from the squamo-columnar junction. The cervix is exposed with a speculum, the excessive mucus is removed by a swab and the end of the spatula is placed into the external os. By turning the spatula fresh cells are scraped from the surface of the cervix. Cotton-tipped applicators have been used to obtain cytological specimens from the cervix[82,109]. Murray[78] developed a modified technique for the collection of cervical material for cyto-diagnosis. He has used a collodion-coated cotton applicator which, after swabbing the cervix, is placed in the fixative solution of ether-alcohol. This dissolves the collodion and separates the material which is then placed on a slide for staining and examination.

The endocervical aspiration smear contains cells that originate from lesions of the endocervix[73,103,109,117]. These might be missed in the surface smear of the cervix. A metal or glass cannula may be used in obtaining aspirations from the endocervical canal[117]. The cannula is inserted into the external os and suction is applied. Clyman[18] has described a cannula made of polyethylene tubing used for aspiration of the endocervical secretions. MacLean[69] has suggested the use of a plastic cervical cap which may be placed over the cervix for 24 hours to collect material for cytological study.

Endometrial aspiration smears are of value in the diagnosis of carcinoma of the uterine fundus[50,51,52,121]. Cary[17] advised the introduction of a metal cannula into the endometrial cavity for this purpose. The polyethylene cannula devised by Clyman[18] has also been used for endometrial aspirations. Hecht[53] has used the Killian antrum cannula for obtaining endometrial aspiration smears. A 10-ml. syringe is used to apply suction. The cervical mucus is removed from the cervical canal and the cervix is cleansed with 1 to 1000 solution of zepharin. The cannula is inserted into the uterine cavity as one does a uterine sound and suction is applied. The suction is released before the cannula is removed so as not to contaminate the specimen with endocervical cells as the tip of the cannula passes through the cervical canal. The surface of the cannula is then wiped clean before the material is expressed onto the slide. A "pure" endometrial aspiration smear may be obtained with this technique. Ayre[8] and MacLean[70] have reported the use of an endometrial brush in obtaining material from the uterine fundus. This brush removes cells from the endometrium by a gentle scraping action and enables one to have fresh cells for cyto-diagnosis.

In mass screening for uterine carcinoma a simple method for collection of adequate material from the genital tract is necessary. In population screening, the patient introduction of a damp

cotton swab in a vaginal applicator of cardboard or rubber tube has been recommended for the collection of material from the vagina[60]. Brunschwig[13] found the Draghi tampon (a tampon covered with nylon) method afforded reliable means in 98.7% of the patients tested for cervical cancer. The material obtained from the tampon left in the vagina overnight appeared to be most satisfactory in collection of the cytological material. Thornton[119] reported an accuracy of 90.5% in the use of tampons for mass screening in cervical cancer. As to the diagnosis of carcinoma of the endometrium, the use of the Draghi tampon has also been found to be effective when the tampon is left in the vagina overnight. Eighty-seven per cent of the smears made by the tampon method from 23 patients with endometrial carcinoma were positive for malignancy (Brunschwig[14]). The smears collected by this method were reviewed by Papanicolaou[88] as to their quality. Dryness of the cells was present but not to the extent one would expect. The tampon method does not provide as adequate material as a routine type of smear but the method does seem satisfactory for mass screening. Scott *et al.*[111] compared the accuracy of diagnosis of the Draghi tampon technique with material obtained by both endocervical aspiration and scraping the surface of the cervix in instances of atypical hyperplasia, carcinoma in situ, invasive carcinoma, and endometrial carcinoma. The tampon method was not as accurate in the diagnosis of atypical hyperplasia or carcinoma in situ. It was satisfactory in the diagnosis of invasive cervical cancer and it appeared to be more so in the diagnosis of carcinoma of the uterine fundus than did the cervical and endocervical smears. Bader *et al.*[9] have shown that the tampon smear compares favorably with the vaginocervical smear in the diagnosis of uterine cancer. Their investigation showed that the optimal time for the tampon to remain in the vagina for collection of material was 1 to 2 hours. Nieburgs[79] reported on the use of a modified menstrual tampon for collection of exfoliated cells from the vagina. The cotton was removed from the upper end of the tampon and was replaced by a nylon sponge. The material collected on the nylon sponge was smeared by drawing the sponge through a box containing two microscopic slides. He found a 100% accuracy by this method after the tampon had been left in the vagina for 4 hours. It was noted that the cells collected by this method seemed larger and the chromatin distribution in the nucleus was less evident. Oppenheim[84] found the collodion-coated applicator developed by Murray to be satisfactory for the collection of material in mass screening.

The material obtained for cytological study by any of the above methods should be expressed on a clean, dry microscopic slide. It should then be spread evenly to form a thin smear. Thick smears do not stain well and are difficult to interpret because of the overlapping of cells. Immediate fixation of the smears in half ether and half 90% alcohol is done to prevent drying. The cells of dry smears become swollen, lose their structural characteristics, and stain unsatisfactorily. The slides should stay in the fixative at least 1 hour before staining, although they may stay in the fixative as long as 2 weeks without bad effects[109]. Papanicolaou and Bridges[89] have suggested a simple method to prevent drying of the smears during mailing. A solution of diaphane is placed over the freshly prepared slide and allowed to dry. This preserves the material and the diaphane can be removed easily by placing the slide in alcohol-ether prep-

aration. Staining can then be carried out in the routine manner.

CARCINOMA OF THE CERVIX. The value of cytology in the diagnosis of malignancy of the cervix has been proven[32,33,34,47,54,75,76,77,91,97]. Reports upon the accuracy of the cytological technique in the diagnosis of cervical cancer have varied from 70% to 98%[1,2,42,46,47,49,54,75]. The smear is a valuable adjunct in the diagnosis of unsuspected invasive carcinoma, carcinoma in situ, and precancerous lesions of the cervix[1,2,5,15,20,29,35,49,61,85,101,107]. A cervix that appears normal on clinical examination may harbor an early cancer and it is in this type of lesion that the cytological technique is of the utmost value. Ayre[7] has demonstrated the "inadequacy of basing the cancer search upon symptomatology." He found 89 (1.69%) cases of proven cancer in 5,278 women having cervical cytology studies. Forty-eight of these cases were early and curable. In the clinically negative group of women the incidence of cancer was 1.3%. In the group with suggestive clinical findings the incidence was only 2.1%. Cancer of the cervix should be detected at a stage when it is curable and this can be accomplished through the use of cytology. In the Memphis study, 108,000 women were screened by the vaginal aspiration smear for uterine cancer[24]. The study resulted in the diagnosis of 373 invasive carcinomas, of which 30% were not suspected, and 393 noninvasive carcinomas, of which 90% were not suspected. Burns and Hartung[15] reported 54 cervical cancers in 6,437 women who were examined and studied cytologically, of which 22 were unsuspected on clinical examination. Reagan and Scott[104] reported 100 cases of squamous cell carcinoma of the cervix of which 14 were detected solely by the cytological technique. Ten of these lesions were intra-epithelial and 4 were invasive. Slate *et al.*[113] found 20 cases of invasive carcinoma, 44 cases of preinvasive carcinoma, and 14 cases of premalignant lesions in 104 positive or suspicious smears among 8,148 private patients. Eighty per cent of the preinvasive lesions and 5% of the invasive lesions would have been missed but for the routine cytological study. Anderson *et al.*[2] detected 45 out of 50 cervical cancers from the study of smears in 3,000 unsuspected cases. Eighty per cent of these were preinvasive cancer. Wrobel[126] found 18 cases of unsuspected cancer in 4,200 women studied by vaginal smears. Ten of these positive cases were early invasive and 8 were in situ. Neiburgs and Pund[80] reported 22 cases of preinvasive cancer among 3,700 women studied by endocervical smears. Malinconico[71] has shown the value of the vaginal smear in diagnosis of early malignant lesions of the cervix in pregnancy. In a cytological study of 680 obstetrical patients, 17 positive smears were found. Seven of these smears reverted to negative in the postpartum course and of the 10 cases that remained positive, 4 were proven to have carcinoma in situ and one invasive carcinoma by biopsy.

The source of the material obtained for cytological study affects the accuracy in diagnosis of cervical carcinoma. Smears from the cervix, either scrapings from the portio or aspirations from the endocervix, give a higher degree of correct positive diagnosis of cervical cancer than do vaginal smears[1,20,85,100,101,117]. The concentration of malignant cells is not as great in vaginal aspirations as they are in smears obtained directly from the cervix. Endocervical aspirations may show malignant cells in the smear preparation when the smear from the surface of the cervix is negative.

Kulcsar's[61] comparative study of vaginal smears and cervical smears in carcinoma in situ showed the vaginal smear to be negative in 45% of the cases and the cervical smear to be negative in only 7% of the cases. Wied[124] obtained cellular material from the vagina, surface of the cervix, and endocervix in cases of clinically evident carcinoma and of subclinical (early invasion or noninvasive) carcinoma. He found the cervical smear to be of more diagnostic value in the clinically evident and the endocervical smear of more value in the subclinical group.

The incidence of false negative smears of cancer of the cervix is about 10%[2,31,42,47,54,57,75,119]. Proper screening of the cytological slide is necessary so as not to overlook an occasional malignant cell that might be present. Misinterpretation of cancer cells, bloody or thick smears, and poor fixation lead to false negative results[19,20]. Malignant cells may degenerate or may not exfoliate and thus not be found in routine smears. The use of the cervical and endocervical smear instead of the vaginal smear alone should decrease the incidence of false negative reports in cervical cancer[61,100,124].

The incidence of false positive smears varies from less than 1% to 5%[2,42,47,54,57,75,99]. Inflammatory changes in exfoliated cells may cause a false positive interpretation of the smear[21,46,57,81]. Trichomonas infection of the lower genital tract causes most atypical cellular changes which may be falsely diagnosed as cancer. Bechtold and Reicher[10] studied atypia in cytological smears of 26 cases of trichomonas infestations. They found that the atypical changes were reversible 2 months after specific therapy for trichomonas vaginalis had been started. Cervical polyps, hyperplasia of the endocervical and endometrial epithelium, misinterpretation of histiocytes and cellular changes incident to pregnancy may cause false positive errors[26,81]. Graham and McGraw[46] reported 70 false positive smears in the study of 8,133 cases. Most of these errors were based on faulty interpretation of histiocytes and atypical inflammatory changes in basal cells and endocervical cells.

The problem of false negative and false positive smears has been summarized as follows: "If criteria for the diagnosis of malignancy were relaxed, the incidence of false negative smears would drop and the test would become more sensitive. However, the rate of false positives would undoubtedly rise and the test would be less specific[46]."

Treatment should never be instituted on the basis of a positive smear without histological confirmation of the cytological findings[5,25]. The clinician should not let himself fall into a state of false security on the basis of a negative smear. A negative smear does not rule out cervical cancer.

It is not always possible to differentiate invasive carcinoma from carcinoma in situ by the cytological technique, however certain changes may be found in cytological smears which suggest noninvasive carcinoma. Papanicolaou[87] has used the term "dyskaryosis" to designate these early changes. "Dyskaryosis" means abnormal nucleus. Dyskaryotic cells may be found in smears of patients with invasive carcinoma. The cytological findings in carcinoma in situ have been described by several authors[87,100,102,105,108]. The nucleus is enlarged, hyperchromatic, and is round or oval. The chromatin pattern may be dense and the nucleus will appear solid, or the chromatin may be arranged in irregular clumps. There is an alteration in the nuclear-cytoplasmic ratio due to the increase in nuclear size. These cells are round or oval, the cytoplasm is well-defined and it may frequently stain orange. Dys-

karyotic cells can be identified as to the squamous epithelium from which they originate (parabasal cell dyskaryosis, intermediate cell dyskaryosis, and superficial cell dyskaryosis). Dyskaryotic cells usually appear as single cells in the smear preparation. The more abnormal malignant cells as the tadpole cell, fiber cell, and the undifferentiated malignant cells are not usually seen in smears of noninvasive cancer.

Cytologists have attempted to identify characteristics in vaginal smears which are precursors of carcinoma in situ. Ayre[6] has used the term "precancer cell complex" to identify these changes. He reported 7 cases of "precancer cell complex" of which 6 developed cancer cells in 6 to 18 months. The precancer cell complex is characterized by an increase of cornified cells and atypical cornified cells with large, multiple, or bizarre-shaped nuclei. The nuclei are hyperactive in appearance, hyperchromatic, and sometimes have a perinuclear halo. Hyperactive basal cells occur frequently. Koss and Durfee[59] reported 4 cases of carcinoma in situ which had been followed by vaginal smears prior to the development of the lesion. None of these smears showed characteristic changes to suggest a premalignant state.

Atypical cervical lesions, as basal cell hyperplasia, may exfoliate dyskaryotic or dysplastic cells. Lapid[62], in the study of the vaginal smears on 2,000 women, found 12 cases of dyskaryosis which on biopsy were not associated with carcinoma in situ. Reagan[105], in a study of carcinoma in situ and dysplastic cells, found that in some instances the dysplasia and carcinoma in situ could be differentiated by the smear method. The dysplastic cells were more likely to be polyhedral rather than round or oval. Cells of dysplasia were larger and more variable in size. The nucleus, which appears granular with widely dispersed particles of chromatin, did not take up as much of the cell area as seen in cells of noninvasive carcinoma. He found that sheets of cells were more frequently seen in the smear of dysplasia than in carcinoma in situ.

All cervical cancers cannot be detected by cytological smears or cervical biopsies. The combination of the smear and the biopsy gives the greatest accuracy in the diagnosis of cervical carcinoma. In early carcinoma of the cervix the biopsy may miss the lesion, especially the one that arises in the cervical canal. The biopsy is limited and shows the histology in only the area from which the specimen was taken. Small biopsy specimens may be cut in the pathological laboratory so as not to show the malignant changes. Smears, on the other hand, give a general sampling of the exfoliated cells from the cervix.

Graham *et al.*[48] in a study of 181 proven cervical cancers, found the first biopsy to be positive in 90% of the cases. The first smear report was positive in 91% of the cases. When the smear and the biopsy were used together, the accuracy of diagnosis of cancer of the cervix was 99%. Daro *et al.*[21] found the smear technique to be more accurate than the biopsy in the diagnosis of cervical cancer. He reported 43 positive smears and 39 positive biopsies among 43 cervical cancers detected in the study of 500 abnormal cervices. Lombard *et al.*[67] reported 47 cases of cervical carcinoma diagnosed by the smear technique in 6,265 women. The first biopsy from 5 of these cancers was negative. Marsh and Hellman[72] found that the smear technique and the multiple biopsy technique gave about the same results in the detection of cancer. In their study the biopsy missed 2 of 4 invasive cervical cancers and the smear missed 6 of 17 noninvasive lesions.

ENDOMETRIAL CARCINOMA. The diag-

nosis of endometrial carcinoma by the cytological technique has not been as accurate as it has in cervical cancer[2,55,99,103,106,110,118,121,125]. Papanicolaou and Traut[92] reported 7 failures in the diagnosis of 53 cases of carcinoma of the fundus by vaginal smear. Graham and Meigs[47] in their early studies reported a 70% accuracy in the diagnosis of carcinoma of the fundus by vaginal and cervical smear. Rakoff[99], in a study of 418 cases, found malignant cells in the vaginal smear of 2 out of 4 carcinomas of the fundus. Scheffey *et al.*[110] reported a 33% error in the diagnosis of carcinoma of the fundus by the cytological technique. Fremont-Smith and Graham[31] reported 20 (20.4%) negative vaginal smears in 98 cancers of the endometrium. Brudenell[12] found 5 false negative smears in cervical scrapings and vaginal aspirations of 16 endometrial cancers. Gusberg[49] reported a 69.8% accuracy, and Anderson *et al.*[2] reported an accuracy of 60% in the diagnosis of carcinoma of the endometrium by the cytological technique.

The malignant characteristics seen in cells of carcinoma of the fundus are not as striking as those of cervical cancer. Exfoliated endometrial cells tend to degenerate before they reach the vagina, to make their identification difficult. The concentration of endometrial malignant cells is not as great in the vaginal and cervical smears as cervical cancer cells. As carcinoma of the endometrium usually is a disease of the postmenopausal woman, malignant cells from the endometrium may not reach the vagina because of atrophic cervical stenosis. Meigs[76] reported 16 negative vaginal smears in 80 cases of carcinoma of the endometrium. The 16 negative slides were reviewed in an attempt to detect overlooked cancer cells. No malignant cells were found in 12 of these cases.

According to Papanicolaou and Marchetti[90] smears from the endocervix and endometrium rather than from the vagina are more accurate in the diagnosis of cancer of the corpus. The introduction of the endometrial aspiration smear has increased the accuracy in the diagnosis of corpus cancer[50,51,52,53,106,121]. Reagan and Schmidt[103] in their study of cervical and endocervical smears found 30 positive smears in 34 cases of endometrial cancer. Reagan and Sommerville[106] compared the value of endocervical aspiration and the endometrial aspiration in the diagnosis of carcinoma of the corpus. Of the 395 patients studied, 20 were proven to have carcinoma among whom the endocervical smear was positive in 14 cases and the endometrial smear in 18 cases. Hecht[53] has reported an accuracy of 92.3% in the use of the endometrial aspiration smear in the diagnosis of corporeal cancer.

The accuracy in the diagnosis of endometrial carcinoma will be increased, as in cervical cancer, if the smear technique is used in conjunction with biopsy. Early cancers of the endometrium may be missed at the time of curettage because of inadequate scrapings or irregularities of the endometrial surface[92]. However, the small lesions usually do exfoliate an abundance of cells for cytological study to enable the diagnosis of fundal cancer by endometrial aspiration when the curettage fails. Hecht[53] reported 6 cases of corpus cancer diagnosed by endometrial aspiration smears but missed on curettage. All 6 of these lesions were confirmed by hysterectomy. Jordan *et al.*[56] compared the accuracy of the vagino-cervical smear, endometrial aspiration smear, and endometrial biopsy in diagnosis of carcinoma of the fundus. The vagino-cervical smear was 68% accurate, the endometrial aspiration 72%, and the

endometrial biopsy 92%. The three studies combined gave an accuracy of 100% in endometrial cancer.

EXTRA-UTERINE MALIGNANCY. Cancer cells may be found in vaginal smears in cases of malignancy of the vulva, bladder, urethra, tubes, ovaries, and other abdominal viscera. If histologic examination of curettings and an adequate cervical biopsy fail to reveal the source of a positive vaginal smear, then an extra-uterine malignancy should be suspected[11,74,115].

The cancer cells originating from the vulva, bladder, and urethra appear in vaginal smears as a contaminant. The study of cells from lesions of the vulva may be an aid in the diagnosis of vulvar carcinoma. Carter[16] studied 18 cases of carcinoma of the vulva by the smear method. His findings show that the most satisfactory smears are made by first removing any exudate from the surface of the lesions and then scraping the lesion with a thin, metal spatula to obtain material for smearing. Fifteen of the 18 cases of vulvar carcinoma could be diagnosed from the smears. A review of the smears from the 3 missed cases showed no malignant cells in 2 instances but carcinoma cells were found in the third case. Smears made by drawing the slide across the vulva lesion are not satisfactory because the cellular elements are distorted or the smears are made up of debris.

Cancer cells which orginate from intra-abdominal, extra-uterine malignancies may be found in secretions of the posterior fornix, even though metastatic lesions to the uterus are not demonstrated. Tumor cells from ovarian cancer, tubal cancer, or any other intra-abdominal malignancies, whether primary or secondary, may pass through the tubes and uterus to appear in the vaginal secretions. Song[115] reported 10 cases of extra-uterine carcinoma in which the vaginal smears were positive, and in which the uterus was free from invasion by the tumor process. The fallopian tubes were secondarily involved by the neoplasm in 5 of these cases. Song has suggested that cancer cells can reach the tube by direct extension, by ascitic fluid which contains tumor cells, or by ameboid motion of the cancer cells. They are then transported through the tubes by ciliary action and peristaltic waves of the tubes to the uterine cavity. From the uterine cavity they find their way to the vagina.

Early clinical diagnosis of carcinoma of the fallopian tubes is always difficult. The cytological technique may be of distinct value in the diagnosis of this lesion. Fidler and Lock[27] found malignant cells in 3 of 4 cases of tubal malignancy studied by cervical smears. Song[114] reported an instance of primary adenocarcinoma of the tube in which malignant cells had been demonstrated in the vaginal secretions. Kernodle *et al.*[57] in the study of 1,709 patients by cervico-vaginal smear found 1 case of tubal carcinoma. Larsson and Schooley[63] reported 2 cases in which vaginal smears were suspicious and carcinoma of the fallopian tube was proven at laparotomy. Brewer and Guderian[11] reported 3 cases of tubal cancer which had atypical or positive smears.

Papanicolaou[87] studied 2 cases of cystoadenocarcinoma of the ovary in which malignant cells were found in the smears from the endometrial cavity. Frech[30] reported a case of adenocarcinoma of the ovary which was detected by vaginal smear. Fidler and Lock[27] reported 7 positive and 4 suspicious cervical smears in 20 patients studied with ovarian cancer. Wachtel and Plester[123], in a survey of 2,550 smears, detected 1 case of ovarian cancer. Umiker and Sheen[120] found malignant cells in vaginal smears of an asympto-

matic patient, who 1 year later was found to have ovarian cancer. Steffan *et al.*[116] reported a patient with positive vaginal smears which proved to be ovarian in origin. Figge and De-Alvarez[28] studied vaginal and cervical smears of 10 patients with ovarian cancer. By the smear technique 7 of these cases were called malignant, and 2 were called suspicious.

Parsons and Taymor[96] reported positive vaginal smears in a patient who had a radical mastectomy 5 years previously. These findings were due to metastasis from the breast lesion to the peritoneal cavity.

RADIATION AND ITS EFFECT ON VAGINAL SMEARS. Radiation causes specific changes in both the normal and malignant cells exfoliated from the genital tract[122]. These may be seen as early as the second day of roentgenologic therapy, and in the case of the normal cells continue to be found some years later. The persistent changes, even years after radiation, may be so atypical that they are confused with malignant cells. Graham[39] described these changes during the course of therapy and shortly thereafter. It may be difficult to determine if an irradiated cell was originally benign or malignant.

As to the nonmalignant cells seen in the vaginal smear the general characteristics of irradiation consist of an increase in leukocytes followed by an increase of histiocytes. The inner and outer basal cells show radiation changes early. These cells become elongated, and the cytoplasm stains brown. The nuclei may become pyknotic or degenerate and appear as discrete black clumps. An increase in the size of the cells to 3 to 4 times normal is a later change, and occurs the tenth day of therapy. The nuclear cytoplasmic ratio remains constant. Vacuolization of the cytoplasm occurs about the fifteenth day and an appearance of bizarre forms is the last change noted. Radiation changes in the precornified cells occur later than in basal cells. These changes are characterized by an increase in size of the cell, vacuolization of the cytoplasm, and infiltration of the cytoplasm by leukocytes. The nuclei become larger, then wrinkled with little structural detail, or pyknotic. Multinucleation, fragmentation, or shrinkage with formation of a perinuclear vacuole may occur. Cornified cells undergo the same changes as the precornified except for the nucleus, which is already pyknotic.

As to the malignant cells radiation changes are characterized by increased cell size, vacuolated cytoplasm, multinucleation and the appearance of giant cells. The nuclei of these cells may appear pyknotic or more frequently washed out and smooth. These changes are more striking in the undifferentiated than in the differentiated cancer cells. In some instances the malignant cells gradually disappear as the treatment progresses or shortly thereafter to suggest sensitivity and therefore a good prognosis.

A special technique for the preparation of smears for study of radiation was suggested by Deden[23]. Smearing of the secretions in the routine manner might cause changes in the cells suggestive of radiation. These cells appear to be more fragile because of their increased viscosity during radiation therapy. A slide should be covered with egg albumen and a drop of vaginal secretion allowed to fall from a pipette and spread over the slide. In this way the cells are not distorted.

CYTOLOGY AND PROGNOSIS. The reports of the use of exfoliated cytology in determining prognosis in cervical cancer have been encouraging. Graham[40, 41], in studying vaginal smears taken during and immediately following radiation therapy, found changes in exfo-

liated cells to serve as an index of response to treatment. She found that radiation changes present in many benign cells indicated a good prognosis. If a good response occurred the malignant cells decreased and gradually disappeared. The correlation of the cytological response with the clinical results gave a prognostic accuracy of 88%.

Graham and Graham[44,45], in later publications, introduced the term radiation reaction (RR) and defined it as "the cell changes in the vaginal smear resulting from radiation." They calculated the percentage of benign cells showing radiation reaction (RR) to determine the prognosis of treatment. In their study they counted 100 benign cells in the post-radiation smears. If 75% or more showed characteristic radiation changes (vacuolization of cytoplasm, increase in size, double nuclei or nuclear changes), it was called a good RR. If less than 60% showed the characteristic changes it was called a poor RR. Of 125 cases of cervical cancer with a 5-year follow-up 63 cases had a good RR and 59% survived 5 years without evidence of disease; 62 cases were classfied as poor RR and only 3% lived 5 years without disease.

Other workers in this field have used the radiation changes in cells as a prognostic index in the treatment of cervical cancer. Nielsen[83] reported a recurrence rate of 8% in patients with good RR and 78% in poor RR. Kohn[58] found radiation reaction to be of value in determining prognosis of cervical cancer. In a series of 50 patients with cervical cancer, he found 32 with a good radiation response and 18 with a poor response. Only 5 of the 32 patients who showed a good RR had persistent disease after therapy whereas 16 of the 18 patients with poor RR showed persistent disease. Shier[112] classified cases that showed 75% or more benign cells with radiation changes as a good response, and 65% or less a poor response. Nineteen (59%) of 32 patients with good response survived 3 years in Stage I and II cervical cancer. One (17%) of the 6 patients with a poor response survived 3 years. If both benign and malignant cells were used in the response study, 85% with good response survived against 9% with poor response.

Prior to therapy, cellular changes which have a prognostic significance have been described in benign cells of the vaginal smear. Graham and Graham[36,44] reported changes in benign cells from untreated cancer patients which indicated a sensitivity to radiation. They introduced the term sensitization response (SR) to denote the presence of these changes by which the response of the tumor to radiation could be predicted. These changes, usually seen in the basal cells, are characterized by lavender staining, increased density of the cytoplasm, and vacuolization and red granules in the cytoplasm. In smears with cornification the basal cells are few, thus a good SR and marked cornification are not found together.

The SR is determined by counting 100 benign epithelial cells in the vaginal smear. If 10% or more show SR changes, it is classified as predicting a good sensitization response. If 9% or less of the 100 normal cells show this change it is classified as poor SR. Graham *et al.*[37] reported a 73% five-year survival rate in cervical cancer treated by radiation with good SR, and only an 18% survival rate in patients found to have poor SR. On the other hand, of 80 patients with poor SR who were treated surgically, 71% survived 2 to 5 years. "It is concluded that SR is a useful prognostic index in patients with cancer of the cervix and that it

may serve in the selection of the appropriate form of treatment."

Graham *et al.*[37] have found the incidence of lymph node metastasis different in marked SR and poor SR groups of Stage I and II cancers. Cases of cervical cancer which showed marked SR had 64% node metastasis and those with poor SR had 17% node metastasis. They have suggested that this may explain why, in their experience, cure rate is higher by radiation therapy than surgery in the marked SR group.

Graham and Goldie[43] compared the size of the unirradiated cornified and precornified cells to that of previously radiated cells. The mean diameter of the former approximated 32 microns while that of the latter was considerably greater. In smears of postirradiated cervical cancer 100 normal cornified and precornified cells were measured. The average diameter was 65 microns or less in 53 cases, of whom 10% survived 5 years. The average diameter was 66 microns or more in 93 cases of whom 48% survived 5 years. They concluded that the degree of increase in cell size following radiation could be used as a prognostic index.

Liu[64] found the incidence of basal cells in the preoperative smear to be of prognostic significance in cervical cancer treated by surgery. Of 12 patients whose preoperative differential count showed 51% or more basal cells, 33% were alive and well. In contrast, of 85 patients whose preoperative smears showed less than 50% basal cells, 44% were alive and well. The prognosis was good in patients with moderate cornification in their preoperative smear which showed an increase of basal cells (more than 20%) following surgery.

Graham and Meigs[38] studied the prognostic significance of malignant cells in vaginal smears following treatment of cervical cancer. Recurrence of a tumor may be recognized by the use of the cytological method long before it is clinically evident. The authors reported 7 treated cases (4 by radiation and 3 by surgery) in which cancer cells were found in the vaginal smear in an average of 14.5 months before it was possible to obtain a positive biopsy. "A positive smear at the end of treatment is an unfavorable prognostic sign. A positive smear at 3 months is regarded with grave suspicion and a positive smear at 6 months undoubtedly means that the treatment has been ineffective." In surgically treated cancer of the cervix "a positive smear after operation indicated disease."

Conclusions. Exfoliative cytology has become an important tool in the detection of gynecological malignancy. This method is most accurate in the diagnosis of cervical cancer. Since the introduction of the endometrial aspiration smear, the accuracy of the cytological technique in corporeal cancer has improved. The cytological method may be helpful in the diagnosis of extra-uterine, abdominal malignancy as cancer cells from such a source can be found in genital smears. The smear should not replace the biopsy but be used in conjunction with it to obtain the greatest accuracy in the diagnosis of genital malignancy. The scope of exfoliative cytology has spread beyond its diagnostic role and its prognostic potentialities show promise.

REFERENCES

(1) **Achenbach, R. R., Johnstone, R. E.,** and **Hertig, A. T.:** Am. J. Obst. & Gynec., **61,** 385, 1951. (2) **Anderson, A. F., Grant, M. P. S., McBryde, R. M.,** and **Cockburn, M. K.:** J. Obst. & Gynec. Brit. Emp., **60,** 345, 1953. (3) **Ayre, J. E.:** Am. J. Obst. & Gynec., **51,** 743, 1946. (4) Idem: Ibid., **53,** 609, 1947. (5) Idem: J. Am.

Med. Assn., **138**, 11, 1944. (**6**) Idem: Am. J. Obst. & Gynec., **58**, 1205, 1949. (**7**) Idem: Obst. & Gynec., **3**, 111, 1954. (**8**) Idem: Ibid., **5**, 137, 1955.

(**9**) **Bader, G. M., Simon, T. R., Koss, L. G.,** and **Day, E.**: Cancer, **10**, 332, 1957. (**10**) **Bechtold, E.,** and **Reicher, N.**: Ibid., **5**, 443, 1952. (**11**) **Brewer J. I.,** and **Guderian, A. M.**: Obst. & Gynec., **8**, 664, 1956. (**12**) **Brudenell, J. M.**: J. Obst. & Gynec. Brit. Emp., **63**, 380, 1956. (**13**) **Brunschwig, A.**: Cancer, **7**, 1182, 1954. (**14**) Idem: Ibid., **10**, 120, 1957. (**15**) **Burns, E. L., Hartung, W. H.,** and **Brittingham, E.**: A.M.A. Arch. Path., **50**, 669, 1950.

(**16**) **Carter, B., Kaufman, L. A.,** and **Cuyler, W. K.**: Surg., Gynec. & Obst., **91**, 600, 1950. (**17**) **Cary, W. H.**: Am. J. Obst. & Gynec., **46**, 422, 1943. (**18**) **Clyman, M. J.**: Obst. & Gynec., **6**, 258, 1955. (**19**) **Cuyler, W. K.**: Am. J. Obst. & Gynec., **62**, 262, 1951.

(**20**) **Dahlin, D. C., Randall, L. M., Soule, E. H.,** and **Dockerty, M. B.**: Surg., Gynec., & Obst., **100**, 463, 1955. (**21**) **Daro, A. F., Rubenstein, M. W.,** and **Gollin, H. A.**: Am. J. Obst. & Gynec., **65**, 364, 1953. (**22**) **Dearing, R.,** and **Liu, W.**: J. Obst. & Gynec. Brit. Emp., **63**, 375, 1956. (**23**) **Deden, C.**: Acta radiol., **43**, 417, 1955.

(**24**) **Erickson, C. C., Everett, B. E., Graves, L. M., Kaiser, R. E., Malmgren, R. A., Rube, I., Schreier, P. C., Cutler, S. J.,** and **Sprunt, D. H.**: J. Am. Med. Assn., **162**, 167, 1956.

(**25**) **Fennell, R. H.,** and **Graham, R. M.**: Cancer, **8**, 310, 1955. (**26**) **Ferguson, J. H.**: Ibid., **2**, 845, 1949. (**27**) **Fidler, H. K.,** and **Lock, D. R.**: Am. J. Obst. & Gynec., **67**, 1103, 1954. (**28**) **Figge, D. C.,** and **DeAlvarez, R. R.**: Obst. & Gynec., **8**, 655, 1956. (**29**) **Foote, F. W.,** and **Li, K. Y. Y.**: Am. J. Obst. & Gynec., **56**, 335, 1948. (**30**) **Frech, H. C.**: Ibid., **57**, 802, 1949. (**31**) **Fremont-Smith, M.,** and **Graham, R. M.**: Surg. Clin. N. America, **27**, 1215, 1947. (**32**) Iidem: J. Am. Med. Assn., **137**, 921, 1948. (**33**) Iidem: Ibid., **150**, 587, 1952. (**34**) **Fremont-Smith, M., Graham, R. M., Jangen, L. T.,** and **Meigs, J. V.**: J. Clin. Endocrinol., **5**, 40, 1945. (**35**) **Fremont-Smith, M., Graham, R. M.,** and **Meigs, J. V.**: New England J. Med., **237**, 302, 1947.

(**36**) **Graham, J. B.,** and **Graham, R. M.**: J. Am. Geriatrics Soc., **3**, 863, 1955. (**37**) **Graham, J. B., Graham, R. M.,** and **Liu, W.**: Surg., Gynec., & Obst., **99**, 555, 1954. (**38**) **Graham, J. B.,** and **Meigs, J. V.**: Am. J. Obst. & Gynec., **64**, 908, 1952. (**39**) **Graham, R. M.**: Surg., Gynec., & Obst., **84**, 153, 1947. (**40**) Idem: Ibid., **84**, 166, 1947. (**41**) Idem: Ibid., **93**, 767, 1951. (**42**) Idem: J. Obst. & Gynec. Brit. Emp., **60**, 483, 1953. (**43**) **Graham, R. M.,** and **Goldie, K. R.**: Cancer, **8**, 71, 1955. (**44**) **Graham, R. M.,** and **Graham, J. B.**: Ibid., **6**, 215, 1953. (**45**) Iidem: Ibid., **8**, 59, 1955. (**46**) **Graham, R. M.,** and **McGraw, J.**: Surg., Gynec., & Obst., **90**, 221, 1950. (**47**) **Graham, R. M.,** and **Meigs, J. V.**: Am. J. Obst. & Gynec., **58**, 843, 1949. (**48**) **Graham, R. M., Sturgis, S. H.,** and **McGraw, J.**: Ibid., **55**, 303, 1948. (**49**) **Gusberg, S. B.**: Ibid., **65**, 1073, 1953.

(**50**) **Hecht, E. L.**: New York J. Med., **52**, 2745, 1952. (**51**) Idem: Am. J. Obst. & Gynec., **64**, 81, 1952. (**52**) Idem: Ibid., **66**, 1321, 1953. (**53**) Idem: Ibid., **71**, 819, 1956.

(**54**) **Isbell, N. P., Jewett, J. F., Allan, M. S.,** and **Hertig, A. T.**: Ibid., **54**, 576, 1947.

(**55**) **Jones, C. A., Neustaedter, T.,** and **Mackenzie, L. L.**: Ibid., **49**, 159, 1945. (**56**) **Jordan, M. J. Bader, G. M.,** and **Nemazie, A. S.**: Obst. & Gynec., **7**, 646, 1956.

(**57**) **Kernodle, J. R., Cuyler, W. K.,** and **Thomas, W. L.**: Am. J. Obst. & Gynec., **56**, 1083, 1948. (**58**) **Kohn, G.**: Acta radiol., **41**, 446, 1954. (**59**) **Koss, L. G.,** and **Durfee, G. R.**: Cancer, **8**, 295, 1955. (**60**) **Kraushaar, O. F., Bradbury, J. T.,** and **Brown, W. E.**: Am. J. Obst. & Gynec., **58**, 447, 1949. (**61**) **Kulcsar, D. D.**: Am. J. Clin. Path., **20**, 958, 1950.

(**62**) **Lapid, L. S.,** and **Goldberger, M. A.**: Am. J. Obst. & Gynec., **61**, 1324, 1951. (**63**) **Larsson, E.,** and **Schooley, J. L.**: Ibid., **72**, 1364, 1956. (**64**) **Liu, W.**: Ibid., **69**, 299, 1955. (**65**) Idem: Cancer, **8**, 779, 1955. (**66**) Idem: Ibid., **10**, 522, 1957. (**67**) **Lombard, H. L., Middleton, M., Warren, S.,** and **Gates, O.**: New England J. Med., **239**, 317, 1948.

(**68**) **Mackenzie, L. L.**: Am. J. Obst. & Gynec., **69**, 629, 1955. (**69**) **MacLean, K. S.**: Science, **114**, 181, 1951. (**70**) Idem: New York State Med. J., **55**, 101, 1955. (**71**) **Malinconico, L. L.**: Am. J. Obst. & Gynec., **73**, 75, 1957. (**72**) **Marsh, M. R.,** and **Hellman, L. M.**: Surg. Clin. N. America, **33**, 525, 1953. (**73**) **McClure, G. W.,** and **Cattell, R. B.**: Ibid., **25**, 550, 1945. (**74**) **McGarvey, R. N.**: Obst. & Gynec., **5**,

257, 1955. (75) **Meigs, J. V.:** Surg., Gynec., & Obst., **81**, 337, 1945. (76) Idem: J. Am. Med. Assn., **133**, 75, 1947. (77) **Meigs, J. V., Graham, R. M., Fremont-Smith, M., Kapnick, I.,** and **Rawson, R. W.:** Surg., Gynec., & Obst., **77**, 449, 1943. (78) **Murray, H.:** Cancer, **7**, 519, 1954.

(79) **Nieburgs, H. E.:** Obst. & Gynec., **7**, 10, 1956. (80) **Nieburgs, H. E.,** and **Pund, E. K.:** Am. J. Obst. & Gynec., **58**, 532, 1949. (81) Iidem: J. Am. Med. Assn., **142**, 221, 1950. (82) **Nieburgs, H. E., Stergus, I., Stephenson, E. M.,** and **Harbin, B. L.:** Ibid., **164**, 1546, 1957. (83) **Nielsen, A. M.:** Acta radiol., **37**, 429, 1952.

(84) **Oppenheim, A., Rosenthal, T.,** and **Modin, M. C.:** J. Am. Med. Assn., **161**, 1067, 1956. (85) **Oxorn, H.,** and **Ayre, J. E.:** Am. J. Surg., **80**, 428, 1950.

(86) **Papanicolaou, G. N.:** Proc. Third Race Betterment Conference. Battle Creek, Michigan: Race Betterment Foundation, p. 528, 1928. (87) Idem: Ann. Int. Med., **31**, 661, 1949. (88) Idem: Cancer, **7**, 1185, 1954. (89) **Papanicolaou, G. N.,** and **Bridges, E. L.:** J. Am. Med. Assn., **164**, 1330, 1957. (90) **Papanicolaou, G. N.,** and **Marchetti, A. A.:** Am. J. Obst. & Gynec., **46**, 421, 1943. (91) **Papanicolaou, G. N.,** and **Traut, H. F.:** Ibid., **42**, 193, 1941. (92) Iidem: Diagnosis of Uterine Cancer by the Vaginal Smear. New York: The Commonwealth Fund, 1943. (93) Idem: New York State J. Med., **43**, 767, 1943. (94) **Papanicolaou, G. N., Traut, H. F.,** and **Marchetti, A. A.:** The Epithelia of Woman's Reproductive Organs. New York: The Commonwealth Fund, 1948. (95) **Parrett, V., Small, C.,** and **Winn, L.:** Am. J. Obst. & Gynec., **56**, 360, 1948. (96) **Parsons, L.,** and **Taymor, M. L.:** Ibid., **66**, 194, 1953. (97) **Peters, H.:** Cancer, **2**, 615, 1949.

(98) **Rakoff, A. E.:** Med. Clin. N. America, **29**, 1546, 1945. (99) Idem: Radiology, **50**, 190, 1948. (100) **Reagan, J. W.:** Cancer, **4**, 255. 1951. (101) Idem: Am. J. Clin. Path., **22**, 231, 1952. (102) **Reagan, J. W.,** and **Hamonic, M. J.:** Cancer, **9**, 385, 1956. (103) **Reagan, J. W.,** and **Schmidt, R. T. F.:** J. Am. Med. Assn., **145**, 82, 1951. (104) **Reagan, J. W.,** and **Scott, R. B.:** Am. J. Obst. & Gynec., **62**, 1347, 1951. (105) **Reagan, J. W., Seidemann, I. L.,** and **Saracusa, Y.:** Cancer, **6**, 224, 1953. (106) **Reagan, J. W.,** and **Sommerville, R. L.:** Am. J. Obst. & Gynec., **68**, 781, 1954. (107) **Rogers, W. S., Ayre, J. E.,** and **Millar, B.:** Obst. & Gynec., **6**, 487, 1955.

(108) **Scapier, J., Day, E.,** and **Durfee, G. R.:** Cancer, **5**, 315, 1952. (109) **Scheffey, L. C.,** and **Rakoff, A. E.:** Med. Clin. N. America, **32**, 1563, 1948. (110) **Scheffey, L. C., Rakoff, A. E.,** and **Hoffman, J.:** Am. J. Obst. & Gynec., **55**, 453, 1948. (111) **Scott, R. B., Brown, A. M.,** and **Reagan, J. W.:** Ibid., **73**, 349, 1957. (112) **Shier, C. B.:** Ibid., **67**, 286, 1954. (113) **Slate, T. A., Martin, P. L.,** and **Merritt, J. W.:** Ibid., **66**, 878, 1953. (114) **Song, Y. S.:** Ibid., **70**, 29, 1955. (115) Idem: Ibid., **73**, 341, 1957. (116) **Steffan, L. J., Weisse, H. A.,** and **Eigenberger, F.:** Ibid., **71**, 1359, 1956. (117) **Studdiford, W. E.,** and **Hecht, E. L.:** Ibid., **67**, 206, 1954. (118) **Swinton, N. W.,** and **Saeli, A. J.:** Surg. Clin. N. America, **34**, 785, 1954.

(119) **Thornton, M. J.:** J. Am. Med. Assn., **159**, 1177, 1955.

(120) **Umiker, W.,** and **Sheen, M.:** Am. J. Obst. & Gynec., **66**, 674, 1953.

(121) **Varangot, J., Granjon, A., Nuovo, V.,** and **Vassy, S.:** Ibid., **68**, 474, 1954. (122) Vincent Memorial Laboratory Staff: The Cytologic Diagnosis of Cancer. Philadelphia: W. B. Saunders Co., 1950.

(123) **Wachtel, E.,** and **Plester, J. A.:** J. Obst. & Gynec. Brit. Emp., **59**, 323, 1952. (124) **Wied, G. L.:** Am. J. Clin. Path., **25**, 742. 1955. (125) **Wiles, J. B.,** and **Hellwig, C. A.:** Ibid., **18**, 283, 1948. (126) **Wrobel, C. J.:** Am. J. Obst. & Gynec., **71**, 402, 1956.

BOOK REVIEWS AND NOTICES

RADIOLOGY OF THE HEART AND GREAT VESSELS. By ROBERT N. COOLEY, M.D., Professor of Radiology, University of Texas Medical Branch, and ROBERT D. SLOAN, M.D., Professor of Radiology, University of Mississippi Medical Center. Pp. 309; 195 ills. Baltimore: Williams & Wilkins Co., 1956. Price, $15.00.

THIS work is a republication in book form of Chapter IV from the three volume looseleaf edition of Golden's textbook "Diagnostic Roentgenology." It presents an admirably concise and readable résumé of present day cardiovascular roentgenology. Ample attention is devoted to the modern technical procedures including laminography, electrokymography and angiocardiography. There is an excellent coverage of the roentgenological aspects of congenital anomalies of the heart and great vessels. Much of the material is based on the authors' experiences when they were members of the Department of Radiology at Johns Hopkins University. The quotations from the literature are well documented and there is an excellent Bibliography. This book can be heartily recommended as a well-balanced consideration of the contribution of Roentgen findings to the clinical study of cardiovascular lesions. H. S.

PEDIATRICS. Edited by DONALD PATERSON, M.D., Formerly Clinical Professor of Pediatrics, University of British Columbia, and JOHN F. MCCREARY, M.D., Professor of Pediatrics, University of British Columbia. Pp. 654; 192 ills. Philadelphia: J. B. Lippincott Co., 1956. Price, $14.00.

THIS new textbook of pediatrics is designed to be intermediate in scope between the comprehensive textbooks which serve as consultants for pediatricians and the smaller handbooks in more or less tabular style which have become rather common in recent years. A textbook in which the discussion of theoretical and controversial points is kept to a minimum in favor of presenting the practical management of most common pediatric problems is in danger of over-simplification or of arbitrariness in point of view. The editors seem very successfully to have avoided these pitfalls. What has emerged is a highly practical and readable text of pediatrics, which attains satisfactorily its goal.

Because the book is frugal in references, and in theory and hypothetical discussion, the student or physician who needs a truly comprehensive survey of his problem will generally need to turn elsewhere; but for the active practitioner with routine problems this volume is recommended as sound and practical. It should find wide favor. V. V.

RECENT PROGRESS IN HORMONE RESEARCH. Edited by GREGORY PINCUS. Pp. 453; illustrated. New York: Academic Press, Inc., 1956. Price, $10.00.

THIS volume contains the proceedings of the twelfth annual Laurentian Hormone Conference which was held at Estes Park, Colorado, in September, 1955. The material is presented in four major sections: I. Hormone Biosynthesis and Metabolism; II. Hormones and Metabolism; III. Pituitary Hormones; and IV. Sex Hormones.

Although many of the discussions are directed to fundamental questions of particular interest to biochemists, physiologists, pharmacologists, and biologists there is also much valuable information for endocrinologists and others concerned with hormonal problems at the clinical level. Among the topics of clinical interest may be mentioned the section on "Hormonal Control of Melanin Pigmentation" by A. B. Lerner and Yi Takahashi; the review of "Pituitary Syndromes in Man" by R. F. Escamilla; the studies of "Testicular Hormone Production" by R. B. Leach *et al.*, and "Aldosterone in Relation to Water and Electrolyte Balance in Man" by J. A. Luetscher, Jr. C. Z.

GESTATION. TRANSACTIONS OF THE THIRD CONFERENCE. Edited by CLAUDE A. VILLEE, PH.D., Associate Professor of Biological Chemistry, Department of Biological Chemistry, Harvard Medical School. New York: Josiah Macy, Jr. Foundation, 1957. Price $4.75.

GESTATION, the Transactions of the Third Conference on Gestation sponsored by the Josiah Macy, Jr. Foundation, is not easy to review because it reports verbatim the informal presentation and discussion of a number of papers which are generally related but

of varied context. The general theme of this particular conference was hormone production during pregnancy and the papers included material as variable as "The Secretions of Various Hormones and the Activity of the Adrenal Cortex in Pregnancy" by Eleanor H. Venning; "Steroidogenesis in Perfused Human Placentas" by Gregory Pincus; "Effects of Hormones on Cellular Metabolism" by Claude A. Villee; "Effect of Fetal Endocrines on Fetal Growth" by Lemen J. Wells and Interrelations of Pituitary Functions and Pregnancy" by Roy O. Greep and others.

The material presented by each participant is generally a review of his current research work rather than a final report of a specific portion of it. The free discussion and questioning by the other conference members, however, usually provides an excellent review of what is known of the topic under discussion. While there is much material in this volume which eventually will influence the practice of obstetrics, the current presentation contains little which will interest the practicing obstetrician. J. W.

Dynamic Psychiatry. By Robert R. Mezer, M.D., Senior Staff Psychiatrist, Community Clinic, Massachusetts Mental Health Center and Harvard Medical School. Pp. 174; 8 ills. New York: Springer Publishing Co., Inc., 1956. Price, $2.50.

This book is useful for those who are called upon to interpret psychiatric concepts in a simple direct fashion. It reflects its development out of lectures to medical students and nurses. It forms a good structure on which can be laid more extensive explanation of theoretical and clinical material. O. E.

The Leukemias: Etiology, Pathophysiology, and Treatment. Edited by J. W. Rebuck, R. W. Monto and F. H. Bethell. Pp. 711; illustrated. New York: Academic Press, Inc., 1957. Price, $13.00.

This volume encompasses the papers presented by some 56 investigators at an international symposium on The Leukemias which was held in Detroit, in March, 1956. The material is grouped under the following headings: 1) The Leukemic Cell, Its Structure and Antigenicity; 2) Genetic and Environmental Factors in the Transmission of Leukemia; 3) Radiation Biology of Leukocytes; 4) The Leukemias and the Malignant Lymphomas; 5) Leukocytic Physiology; 6) Metabolism of Amino and Nucleic Acids in the Leukemias; 7) Further Biochemical Considerations in the Leukemias; and 8) Nucleic Acids as the Target for Chemotherapy: Mechanisms of Drug Action and Resistance.

Aside from the generally excellent papers, there are also included many of the valuable comments and discussions which were made during the proceedings.

This comprehensive volume is a "must" for all hematologists and is highly recommended to anyone concerned with leukemia whether at a basic or clinical level. C. Z.

Fundamentals of Clinical Fluoroscopy with Essentials of Roentgen Interpretation. By Charles B. Storch, Associate Attending Roentgenologist, Beth-El Hospital, Brooklyn, N.Y. 2nd ed. Pp. 305; 318 ills. New York: Grune & Stratton, Inc., 1956. Price, $8.75.

Relatively minor changes have been made in the second edition of this useful introduction to clinical fluoroscopy. The author wisely emphasizes the hazards, as well as the diagnostic limitations, of fluoroscopy as an independent technique if divorced from adequate complementary radiographic studies. Unfortunately, since fluoroscopy presents the greatest radiation exposure danger to both patient and physician of any diagnostic Roentgen-ray procedure, it is to be feared that the casual and occasional reader of this book will omit or be unimpressed by the brief introductory chapter covering dangers, radiologic protection and dark adaptation. H. S.

The Person Behind the Disease. By Julius Bauer, M.D., Clinical Professor of Medicine, College of Medical Evangelists. Pp. 136; 5 ills. New York: Grune & Stratton, Inc., 1956. Price, $3.50.

The author undertakes in this small volume "to elaborate on the fundamental principles of a holistic concept of medicine." As might be expected, attention is drawn to the uniqueness of the individual, various pathogenic and nonpathogenic genetic patterns, "constitutional biologic organ inferiority," and the need for treatment of the entire individual rather than only a part.

This is one of several recent publications which reflect the current revival of interest in "comprehensive medicine." Accordingly, it should be of interest to those responsible for medical education, both at the administrative and teaching level. C. Z.

DIABETES MELLITUS WITH EMPHASIS ON CHILDREN AND YOUNG ADULTS. By T. S. DANOWSKI, M.D., Renziehausen Professor of Research Medicine, University of Pittsburgh. Pp. 510; 177 ills. Baltimore: The Williams & Wilkins Co., 1957. Price, $13.50.

AMONG the current texts on diabetes mellitus there are none presenting the fundamental concepts of intermediary metabolism with the clarity and thoroughness as that of Dr. Danowski. The biochemical and hormonal aspects of diabetes are analyzed in Part I with descriptions of protein, fat, and carbohydrate metabolism as influenced by endocrine and other factors. Mechanisms of action of the adrenal steroids, thyroid hormone, and diabetogenic pituitary hormones are discussed in relation to the biochemistry of cellular activity. In the section entitled Manifestations, Diagnosis and Treatment, the emphasis is directed largely toward the author's experience with the juvenile diabetic group. Much of this material is equally pertinent to the treatment of the adult, stable or unstable diabetic patient. The description of insulin hypoglycemia is too brief and is devoid of discussion of the precipitating factors, prevention and treatment. Also lacking is a section on the pathology of diabetes. An interesting and enlightened survey of the personality and emotional response of diabetic patients is reviewed in the final section dealing with developmental aspects and complications.

The excellence of this text attests to the wide clinical experience and research activities of the author. It is highly recommended for physicians engaged in the treatment of diabetic patients of any age. C. S.

CIBA FOUNDATION SYMPOSIUM ON PAPER ELECTROPHORESIS. Edited by G. E. W. WOLSTENHOLME AND E. C. P. MILLAR, Ciba Foundation. Pp. 224; 70 ills. Boston: Little, Brown & Company (London: J. and A. Churchill, Ltd.), 1956. Price, $6.75.

THIS volume consists of papers presented at a symposium held in London, 27–29 July, 1955. Each paper was followed by rather complete discussions by various members invited to the meeting. A number of short communications were included in addition to the more detailed presentations. Among the latter were the following:

"General Methods of Paper Electrophoresis with Examples of its Use in Medical and Biochemical Problems" by W. Grassmann.

"Evaluation of the Albumin-globulin Ratio of Blood Plasma or Serum by Paper Electrophoresis" by E. M. Abdel-Wahab, V. H. Rees and D. H. R. Laurence.

"Analysis of Human Haemoglobins by Paper Electrophoresis" by J. C. White, G. H. Beaven and M. Ellis.

"A Paper Electrophoresis Study of the Effect of ACTH and Cortisone on the Protein-bound Serum Polysaccharides in Some Patients with Rheumatoid Arthritis, Ulcerative Colitis," by H. Laurell.

"Physicochemical Aspects and their Relationship to the Design of Apparatus" by H. Svensson.

"Analysis of Separated Materials I" by E. M. Crook.

"Analysis of Separated Materials II" by N. H. Martin.

"The Future of the Technique in its Application to Clinical Research and Routine Analysis" by E. L. Durrum.

There was a general discussion prior to the last paper in which various participants took part. Among the short communications some of the technical and practical aspects of paper electrophoresis were discussed. These included descriptions of such problems as the relationship of areas to concentrations and the difficulties met in attempts to obtain consistently good dyes. High voltage electrophoresis was also described.

This volume is richly illustrated with many excellent photographs of apparatus and electrophoretic patterns. It should be of considerable value to one who contemplates the use of this technique and of much interest to those now engaged in this activity. R. B.

LUPUS NEPHRITIS: A CLINICAL AND PATHOLOGICAL STUDY BASED ON RENAL BIOPSIES. By ROBERT C. MUEHRCKE, M.D., ROBERT M. KARK, M.D., CONRAD L. PIRANI, M.D., AND VICTOR E. POLLAK, M.B., M.R.C.P.E. Pp. 145; 43 ills., and 10 color plates. Baltimore: The Williams & Wilkins Co., 1957. Price, $3.00.

FROM study of a wealth of clinical, laboratory, and pathologic material the authors have written an excellent treatise on the kidney changes associated with systemic lupus erythematosus. Chapters are devoted to a review of the clinical features, renal pathology, the etiology and pathogenesis of the renal lesions, and then to the authors' own findings. The problems of treatment of lupus nephritis, its prognosis, and possible relation-

ship to other diseases are discussed. The volume is concluded with an important chapter on the natural history of lupus nephritis.

This monograph is recommended to clinicians and pathologists who are concerned with this serious and difficult problem.

C. Z.

NEW BOOKS

Instrumentation in Bio-Medical Research Publication 472. By Paul E. Klopsteg, Consultant to the Biology Council. Pp. 14. Washington: National Academy of Sciences-National Research Council, 1957.

This report calls attention to the increasing use and complexity of instruments employed in medical research, the need for more effective and efficient use of such instruments, and presents recommendations for dealing with this problem. This important pamphlet should be read by everyone concerned with research in biology and medicine. C. Z.

The Liver: Some Physiological and Clinical Aspects. British Medical Bulletin, Vol. 13, No. 2, May, 1957. Pp. 77; 23 ills. London: Medical Department of the British Council, 1957. Price, $3.25.

A group of interesting papers summarizing the current knowledge of liver function and the pathological and clinical aspects of liver disease. R. K.

The Changing Patient-Doctor Relationship. By Martin G. Vorhaus, M.D. Pp. 310; illustrated. New York: Horizon Press, Inc., 1957. Price, $3.95.

There are many books these days on the doctor-patient relationship. This one is different, and should fill a real need in the lives of those within the profession who would like to understand more without taking time for a course.

This book has chapters devoted to "The Patient and How He Became One." Likewise there is a chapter on the doctor and how he became one. The author discusses such things as accepting responsibility and acquiring empathy, and then goes on to describe the tools of human understanding.

Following these introductory chapters the book has some excellent case history presentations, illustrating how the author deals with the patient in cases of duodenal ulcer, obesity, hypertension and colitis. This material is very useful, nearly as good as looking over the doctor's shoulder. O. E.

The Pharmacology of Psychotomimetic and Psychotherapeutic Drugs. By Seymour S. Kety, and 55 others, National Institutes of Health. Pp. 433; 151 ills. New York: New York Academy of Sciences, 1957. Price, $5.00.

This is a timely publication comprising contributions from 56 well known, highly qualified investigators. It contains a total of 33 papers presented at a conference sponsored by the New York Academy of Sciences in April, 1956. Psychopharmacology is a rapidly advancing field and, although still in an early experimental stage, the widespread use of ataractic drugs emphasizes the necessity for the practitioner, as well as the investigator, to keep abreast of this important field. This volume provides one opportunity for them to do so. R. S.

Les Nouveaux Syndromes Hemorragiques La Dysprothrombie. By P. Chevallier and A. Fiehrer. Pp. 128; 25 ills. Paris: Masson et Cie, 1957. Price, 1.500 fr.

This small monograph, written in French, deals with various hemorrhagic states associated with disturbances in the prothrombin mechanism. Clinical, diagnostic, and treatment features of both primary and secondary prothrombin disorders are discussed.

Clinicians in general and hematologists in particular will find this a concise and current summary of the subject. C. Z.

Psychiatric Aspects of School Desegregation. Report No. 37. Pp. 94. New York: Group for the Advancement of Psychiatry, 1957. Price, $1.00.

This report No. 37 of the Group for the Advancement of Psychiatry is a careful analysis on the part of an interdisciplinary committee of the various factors which enter into this complex social problem. It discusses the functions of racial myths and prejudices as well as the psychodynamics of changing attitudes. It discusses group processes and the role of Authority in changing attitudes. There is a section on the responses of various groups to desegregation including the educator and parent. There is an annotated reading list. .It is a brief, concise, up-to-date, and informative report. O. E.

Clinical Pathology in General Practice. From the British Medical Journal. Pp. 321; 45 ills. Philadelphia: J. B. Lippincott Co., 1957. Price, $5.00.

This volume consists of a series of articles which appeared originally in the British Medical Journal. They are concerned with various laboratory procedures used in general practice in Great Britain. The first few chapters describe practical techniques such as venipuncture, lumbar puncture, biopsies, and "postmortem technique for the occasional performer." Other sections deal with renal and gastrointestinal tests, procedures to be followed in various infections, hematology, metabolic and endocrine disorders, skin and tropical diseases, and industrial poisoning. Although some of the chapters would be limited in interest to British physicians, there

is considerable material which would be helpful to general practitioners anywhere. C. Z.

Mercury and Its Compounds. By Cecil V. King, Harry Gold, Harold Wolff and 30 others, New York University. Pp. 295; 87 ills. New York: New York Academy of Sciences, 1957. Price, $3.50.

This volume, comprising 23 papers by 33 authors, is a useful reference to many of the properties and uses of mercury. It is divided into three parts as follows: I) Physics and Chemistry, II) Pharmacology and Toxicology, and III) Clinical Medicine. R. S.

The Riddle of Stuttering. By C. S. Bluemel, M.D. Pp. 142; 9 ills. Danville, Illinois: The Interstate Publishing Company, 1957. Price: cloth bound copy, $3.50; paper bound copy, $1.50.

Over a period of years the author has contributed to the literature on stuttering and in this book, he attempts to reformulate his concepts concerning the problem based on psychiatric theory. Stuttering is described as nonorganized speech in the young child whereas stammering is disorganized speech. Because of predisposing personality traits, certain children tend to become stammerers under stress. Bluemel feels that therapy for stammering should not be directed primarily to the speech disorder. He is concerned with development and training of good mental patterns of speech since he feels that talking is thinking out loud. The answer to the riddle of stuttering seems no closer at hand as a result of this book. M. H.

Regulation and Mode of Action of Thyroid Hormones. Edited by G. E. W. Wolstenholme, O.B.E., M.A., M.B., B.Ch., and Elaine C. P. Millar, A.H.-W.C., A.R.I.C. Ciba Foundation Symposium. Pp. 311; 114 ills. Boston: Little, Brown & Company, 1957. Price, $8.50.

This interesting collection of contributions by leading students in the field of thyroid gland function presents the most up-to-date information available on the subject. It will be of particular importance to workers in the field; its value to clinicians at large is somewhat limited, but will be of interest in terms of pointing the direction towards which investigations into the intricacies of thyroid function are proceeding. W. P.

Symposium über Arteriosklerose/ sur l'artériosclerose/ on arteriosclerosis. Bulletin der Schweizerischen Akademie der Medizinischen Wissenschaften, Special Number. Pp. 488; illustrated. Basel: Benno Schwabe & Co., 1957.

This symposium held in August, 1956, starts with the lecture of W. Dock: "Are there effective ways of managing arteriosclerosis?" The first main topic deals with blood vessel factors and arteriosclerosis, morphology, hemodynamics, etiology and pathogenesis (13 papers). The second main topic is devoted to metabolism and arteriosclerosis, the subdivisions being blood lipids and blood lipoproteins, dietary factors, influence of vitamins and hormones (24 papers). And the third main topic is devoted to clinic and therapy (7 papers). The papers were given in four languages and each paper has a detailed summary in English, German, and French, and most of them give detailed references. Discussions follow. The contributions are of exceptionally high quality, most of them represent original work, and to the student of this all-important topic, with all its many ramifications, this volume is highly recommended. H. R.

Meprobamate and Other Agents Used in Mental Disturbances. By James G. Miller and Frank M. Berger and 43 others. Ann. New York Acad. Sci., **67**, Art. 10, pp. 671–894. New York: New York Acad. Sci., 1957. Price, $4.00.

Unstable Chemical Species: Free Radicals, Ions, and Excited Molecules. By Thomas Freund and Henry C. Thacher, Jr., and 20 others. Ann. New York Acad. Sci., **67**, Art. 9, pp. 447–670. New York: New York Acad. Sci., 1957. Price, $4.00.

Viruses in Search of Disease. By Robert J. Huebner and 38 others. Ann. New York Acad. Sci., **67**, Art. 8, pp. 209–446. New York: New York Acad. Sci., 1957. Price, $5.00.

Group Psychotherapy: The Psychoanalytic Approach. By S. H. Foulkes, Consultant in Psychiatry at Behtlem Royal and Maudsley Hospital and E. J. Anthony, Senior Lecturer in Child Psychiatry, University of London: Pp. 263; 17 ills. Baltimore: Penguin Books, Inc., 1957. Price, 85 cents.

The group analysts of Britain, unlike American workers, did not consider psychoanalytic concepts sufficient for the practice of group analysis. There the psycho-social concepts of Kurt Lewin and the sociometry of J. L. Moreno have been turned to good advantage. This work presents not only an unusually lucid account of the psychotherapeutic group in terms of group dynamics from the psychoanalytic view, but also includes a survey of related methods. M. B.

Second Tissue Homotransplantation Conference. By John Marquis Converse, Blair O. Rogers and 47 others. Pp.

338; 198 ills. New York: The New York Academy of Sciences, 1957. Price, $4.50.

This paper-bound book has limited interest, because of its restricted and highly specialized subject matter. The volume is composed of a group of papers presented at the Second Tissue Homotransplantation Conference and is mainly concerned with the basic aspects of tissue grafting. This book is written primarily for the plastic surgeon. The introductory remarks by Hilary Koprowski and the introduction by John Marquis Converse and Blair O. Rogers give the theme of the several presentations. A. P.

Report of Study Group of Atherosclerosis and Ischaemic Heart Disease. WHO Technical Report Series No. 117. Pp. 40. Geneva: World Health Organization, 1957. Price, $.30.

NEW EDITIONS

Gifford's Textbook of Ophthalmology. By Francis Heed Adler, M.D., Professor of Ophthalmology, University of Pennsylvania School of Medicine. 6th ed. Pp. 483; 277 ills. and 26 color plates. Philadelphia: W. B. Saunders Co., 1957. Price, $8.00.

This text is written for medical students and general practitioners. It is written in clear style and is easily understandable. The contents have been well chosen. There are numerous improvements over the fifth edition. It does much to convey to physicians the importance and fascinations of ophthalmology. It is an excellent textbook. G. G.

Hutchison's Clinical Methods. By Donald Hunter, M.D., F.R.C.P., Physician to London Hospital and R. R. Bomford, D.M., F.R.C.P., Physician to London Hospital. 13th ed. Pp. 452; 120 ills., some in color. Philadelphia: J. B. Lippincott Co., 1957. Price, $6.00.

Thirteen editions in sixty years since its first appearance testify to the value of this epitome of diagnostic methods including physical examination and the essential laboratory tests. R. K.

Differentialdiagnose Innerer Krankheiten. By Prof. Dr. Robert Hegglin, University of Zurich. 5th ed. Pp. 749; 459 ills., many in color. Stuttgart: Georg Thieme Verlag, 1957 (Intercontinental Medical Book Corporation, New York, U.S. Agents). Price, $18.95.

An excellent text on the differential diagnosis of internal diseases, that has proved exceedingly popular as shown by five editions in as many years. The presentation is clear-cut, concise and supported by numerous good illustrations. Internists and general practitioners, who read German, will find this a useful text. R. K.

Psychosomatic Medicine. By Edward Weiss, M.D., Professor of Clinical Medicine, Temple University School of Medicine, and O. Spurgeon English, M.D., Professor and Head of the Department of Psychiatry, Temple University School of Medicine. 3rd ed. Pp. 557; 8 ills. Philadelphia: W. B. Saunders Co., 1957. Price, $10.50.

Physicians will welcome the third edition of this excellent and unique medical text which has appeared in Spanish, Portuguese and French translations. Much of the present volume has been re-written in conformity with newer knowledge and much new clinical material has been added. R. K.

Dorland's Illustrated Medical Dictionary. Edited by Leslie Brainerd Arey, Ph.D., Sc.D., LL.D., William Burrows, Ph.D., J. P. Greenhill, M.D., and Richard M. Hewitt, A.M., M.D. 23rd ed. Pp. 1,598; 700 ills. and 50 plates. Philadelphia: W. B. Saunders Co., 1957. Price, $12.50.

The 23rd edition of this outstanding and standard work has a new name: Dorland's Illustrated Medical Dictionary to conform with the name by which it has become familiarly known to the generations of physicians who have used it. The editors have continued to show excellent judgment in revision, addition of new material, re-arrangement, and the problems involved in keeping the book within reasonable size. R. K.

Diagnosis and Treatment of Diseases in the Tropics. By H. C. Trowell, O.B.E., M.D. (Lond.), F.R.C.P., Senior Specialist, Mulago Hospital. 3rd ed. Pp. 332; 37 ills. London: Baillière, Tindall and Cox, 1956 (Williams & Wilkins Co., Baltimore, U.S. Agents). Price, $3.75.

From the Uganda Medical School is appearing a series of pocket-sized medical texts for the use of students in tropical and sub-tropical countries. This volume, in its third edition, attempts to cover so wide a range in so small a compass that its usefulness is quite limited. R. K.

Textbook of Medicine. Edited by Sir John Conybeare, K.B.E., M.C., D.M.(Oxon.), F.R.C.P., Consulting Physician to Guy's Hospital, London, and W. N. Mann,

M.D. (LOND.), F.R.C.P., Physician to Her Majesty's Household, Physician to Guy's Hospital. 12th ed. Pp. 859; 71 ills. London: E. & S. Livingstone, Ltd., 1957 (Williams & Wilkins Company, Baltimore, U.S. Agents). Price, $9.00.

TWELVE editions since the first publication of this text in 1929 attest to the popularity of this practical volume. The supject matter is concise and thanks to good editing and not too many contributors (there are nineteen), there is good coverage without significant duplication or omission. The format is convenient and the illustrations are excellent. The Index is, unfortunately, far too brief. The book should find a wide approval. R. K.

Emergencies in Medical Practice. Edited by C. ALLAN BIRCH, M.D., F.R.C.P. 5th ed. Pp. 684; 155 ills., 12 in color. Edinburgh and London: E. & S. Livingstone Ltd., 1956 (Williams & Wilkins Company, Baltimore, U.S. Agents). Price, $8.00.

AN excellent concise presentation of the many aspects of emergencies in medical practice. It clearly merits the popularity that has called for five editions in nine years and translations in German and Spanish. R. K.

A Manual of the Common Contagious Diseases. BY PHILIP MOEN STIMSON, A.B., M.D., Professor of Clinical Pediatrics, Cornell University Medical College, and HORACE LOUIS HODES, A.B., M.D., Pediatrician-in-Chief, Mt. Sinai Hospital, New York. 5th ed. Pp. 624; 84 ills., and 10 plates, 8 in color. Philadelphia: Lea & Febiger, 1956. Price, $8.50.

THE profession will welcome this new edition of a standard text that has been popular since its first appearance in 1931. The senior author, Doctor Stimson, has associated with him a capable worker and teacher in this field, Doctor Hodes, of the Medical Faculty of the College of Physicians and Surgeons of Columbia University. R. K.

THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES

Published Monthly. Illustrated.

Subscription price: 1 year $10.00; 2 years $18.00.
(One year subscription to Canada, South and Central America, Philippines and Spain, $10.36; all other countries $11.14.)

All foreign subscriptions payable in advance in United States dollars.
Business Correspondence: Lea & Febiger, 600 S. Washington Sq., Philadelphia 6, Pa.

NOTICE AND INSTRUCTIONS TO CONTRIBUTORS

MANUSCRIPTS intended for publication in THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES, and correspondence should be sent to the Editor:

DR. RICHARD A. KERN, Temple University School of Medicine, Philadelphia 40, Pa.

Articles are accepted for publication in THE AMERICAN JOURNAL OF THE MEDICAL SCIENCES exclusively, except in the case of subsequent publication in Society proceedings.

MANUSCRIPTS should be typewritten on one side only, of good quality paper and should be double spaced with liberal margins. The author's chief position and, when possible, the Department from which the work is produced should be indicated in the subtitle.

ILLUSTRATIONS accompanying articles should be numbered and have typed captions bearing corresponding numbers. For identification they should also have the author's name written on the margin or back. Line drawings and charts should be prepared with black India ink. Special arrangements can be made for printing color plates, elaborate tables or an excessive number of illustrations.

REFERENCES should be numbered and at the end of the articles arranged alphabetically according to the name of the first author and should be complete, that is, author's name, journal, volume, page and year (in Arabic numbers).

RETURN POSTAGE should accompany all manuscripts but will be returned to the author if the manuscript is accepted.

100 REPRINTS will be supplied gratis. Covers will be omitted on all articles. Orders of additional reprints will be supplied in multiples of 100. An order form showing cost of reprints is sent with proof. Individual reprints can usually be obtained by writing directly to the author.

Index photographed at the
beginning for the convenience
of the microfilm user.

DOES NOT CIRCULATE